137761

STIC-Biotech/ChemLib

From: McElwain, Elizabeth
Sent: Monday, November 15, 2004 10:48 AM
To: STIC-Biotech/ChemLib
Subject: search

Please search for 09/991,152
The broadest claim is drawn to a plant or bacteria transformed with transgenes encoding
1) a 3-hydroxyacyl-ACP thioesterase; and
2) a 3-hydroxyacyl CoA synthetase
wherein, a medium chain length PHA accumulates.

Thank you,
Beth

Elizabeth F. McElwain, Ph.D.
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571-272-0802
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****
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Searcher: P. Schwiebert
Searcher Phone: 2-2526
Date Searcher Picked up: 11/24
Date Completed: 11/29
Searcher Prep/Rev. Time: 18
Online Time: 45

****
Type of Search
NA Sequence: #
AA Sequence :#
Structure: #
Bibliographic: ✓
Litigation:
Patent Family:
Other:

****
Vendors and cost where applicable
STN: 190.33
DIALOG:
QUESTEL/ORBIT:
LEXIS/NEXIS:
SEQUENCE SYSTEM:
WWW/Internet:
Other(Specify):

STIC Search Report
Biotech-Chem Library

STIC Database Tracking Number: 137761

TO: Elizabeth McElwain **Location: REM-2A11&2C18** **Art Unit: 1638** **Monday, November 29, 2004** **Case Serial Number: 09/991152**	**From: David Schreiber** **Location: Biotech-Chem Library** **Remsen E01A61** **Phone: 272-2526** **david.schreiber@uspto.gov**

Search Notes

ls 1

FILE 'MEDLINE, HCAPLUS, BIOSIS, EMBASE, WPIDS, SCISEARCH, AGRICOLA'
NTERED AT 10:48:03 ON 29 NOV 2004)
46 DUP REM L21 (72 DUPLICATES REMOVED)

ue 122
8 SEA AQUIN S?/AU
184 SEA PEOPLES O?/AU
713 SEA SNELL K?/AU
885 SEA (L1 OR L2 OR L3)
7 SEA L4 AND THIOESTERASE?
5 SEA L5 AND SYNTHETASE?
3 SEA L6 AND CHAIN(3A) LENGTH?
541 SEA ACP(3A) THIOESTERASE?
60 SEA (HYDROXYACYL OR HYDROXY(A) ACYL)(3A) SYNTHETASE?
2 SEA L8 AND L9
10943 SEA (COA OR CO(A) A OR COENZYME(A) A)(3A) SYNTHETASE?
12 SEA L8 AND L11
84 SEA L8 AND CHAIN(3A) LENGTH?
34 SEA L13 AND MEDIUM(3A) CHAIN
2208 SEA MEDIUM(3A) CHAIN(3A) LENGTH?
629 SEA L16 AND PHA
513 SEA L16 AND POLYHYDROXYALKANOATE?
697 SEA L17 OR L18
76 SEA (TRANSGEN? OR TRANSFORM? OR TRANSFECT?) AND L19
118 SEA L7 OR L10 OR L12 OR L15 OR L20
46 DUP REM L21 (72 DUPLICATES REMOVED)

ll 122 1-46

NSWER 1 OF 46 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on
TN
004:360016 SCISEARCH
he Genuine Article (R) Number: 811BR
n the export of fatty acids from the chloroplast
oo A J K; Ohlrogge J B; Pollard M (Reprint)
ichigan State Univ, Dept Plant Biol, E Lansing, MI 48824 USA (Reprint)
SA
OURNAL OF BIOLOGICAL CHEMISTRY, (16 APR 2004) Vol. 279, No. 16, pp.
6101-16110.
ublisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE
IKE, BETHESDA, MD 20814-3996 USA.
SSN: 0021-9258.
rticle; Journal
nglish
eference Count: 54
The model for export of fatty acids from plastids proposes that the
cyl-ACP (acyl carrier protein) product of de novo fatty acid synthesis
s hydrolyzed in the stroma by acyl-**ACP thioesterases**
nd the free fatty acid (FFA) released is then transferred to the outer
nvelope of the plastid where it is reactivated to acyl-CoA for
tilization in cytosolic glycerolipid synthesis. Experiments were
erformed to assess whether the delivery of nascent FFA from the stroma
or long chain acyl-CoA synthesis (LACS) occurs via simple diffusion or a
ore complex mechanism. The flux through the in vivo FFA pool was
stimated using kinetic labeling experiments with spinach and pea leaves.
he maximum half-life for FFA in the export pool was less than or equal to

1 s. Isolated pea chloroplasts incubated in the light with [C-14] acetate gave a linear accumulation of FFA. When CoASH and ATP were present there was also a linear accumulation of acyl-CoA thioesters (plus derived polar lipids), with no measurable lag phase (< 30 s), indicating that the FFA pool supplying LACS rapidly reached steady state. The LACS reaction was also measured independently in the dark after in situ generated FFA had accumulated yielding estimates of LACS substrate-velocity relationships. Based on these experiments the LACS reaction with in situ generated FFA as substrate is only about 3% of the LACS activity required in vivo at the very low concentrations of the FFA export pool calculated from the in vivo experiment. Furthermore, bovine serum albumin rapidly removed in situ generated FFA from chloroplasts, but could not compete effectively for "nascent" FFA substrates of LACS. Together the data suggest a locally channeled pool of exported FFA that is closely linked to LACS.

CC BIOCHEMISTRY & MOLECULAR BIOLOGY

STP KeyWords Plus (R): ACYL-**COA SYNTHETASE**; **COENZYME-A SYNTHETASE**; BOVINE SERUM-ALBUMIN; OUTER-MEMBRANE; SACCHAROMYCES-CEREVISIAE; GLYCEROLIPID METABOLISM; PHOSPHOLIPID-BILAYERS; SPINACH-CHLOROPLASTS; ENVELOPE MEMBRANES; TRANSPORT PROTEIN

RE

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)
ABUMRAD N	1998	39	2309	J LIPID RES
ANDREWS J	1983	72	735	PLANT PHYSIOL
ARNON D I	1949	24	1	PLANT PHYSIOL
BAO X M	2000	22	39	PLANT J
BLOCK M A	1983	153	377	FEBS LETT
BROWSE J	1981	196	347	BIOCHEM J
BRUCE B D	1994		1	PLANT MOL BIOL MANUA
COE N R	1999	274	36300	J BIOL CHEM
DIRUSSO C C	1999	192	41	MOL CELL BIOCHEM
DIRUSSO C C	1999	38	129	PROG LIPID RES
DOUCE R	1990	6	173	ANNU REV CELL BIOL
DOUCE R	1982		239	METHODS CHLOROPLAST
ENGESETH N J	1996	331	55	ARCH BIOCHEM BIOPHYS
FLUGGE U I	1984	169	85	FEBS LETT
GARDINER S E	1984	74	890	PLANT PHYSIOL
HAMILTON J A	1984	81	3718	P NATL ACAD SCI USA
HAMILTON J A	1998	39	467	J LIPID RES
HARA A	1978	90	420	ANAL BIOCHEM
HEBER U	1981	32	139	ANNU REV PLANT PHYS
HEINZ E	1983	72	273	PLANT PHYSIOL
HETTEMA E H	2000	1486	18	BBA-MOL CELL BIOL L
HETTEMA E H	1996	15	3813	EMBO J
JOHNSON P E	2002	215	515	PLANTA
JOYARD J	1981	67	250	PLANT PHYSIOL
KAHN M U	1977	140	179	J CHROMATOGR
KAMP F	1995	34	11928	BIOCHEMISTRY-US
KAMP F	2003	278	7988	J BIOL CHEM
KAMP F	1993	32	11074	BIOCHEMISTRY-US
KEEGSTRA K	1986	118	316	METHOD ENZYMOL
KLEINFELD A M	1997	36	14140	BIOCHEMISTRY-US
KLEINFELD A M	1998	37	8011	BIOCHEMISTRY-US
KOO A J K	2002	130	823	PLANT PHYSIOL
KUMAR G B	1993	268	15469	J BIOL CHEM
LILLEY R M	1975	75	1	NEW PHYTOL
MANGROO D	1992	267	17095	J BIOL CHEM

OHLROGGE J B .	1979	76	1194	P NATL ACAD SCI USA
OHLROGGE J	1995	7	957	PLANT CELL
PARKS J S	1983	258	9262	J BIOL CHEM
POLLARD M	1999	121	1217	PLANT PHYSIOL
ROUGHAN P G	1977	162	457	BIOCHEM J
ROUGHAN P G	1982	33	97	ANNU REV PLANT PHYS
ROUGHAN P G	1987	148	327	METHOD ENZYMOL
ROUGHAN P G	1980	18	221	PLANT SCI LETT
ROUGHAN P G	1980	188	17	BIOCHEM J
ROUGHAN P G	1996	110	1239	PLANT PHYSIOL
ROUGHAN P G	1979	184	193	BIOCHEM J
ROUGHAN P G	1981	135	182	FEBS LETT
SCHAFFER J E	1994	79	427	CELL
SCHNURR J A	2002	129	1700	PLANT PHYSIOL
SHINE W E	1976	172	110	ARCH BIOCHEM BIOPHYS
SHOCKEY J M	2002	129	1710	PLANT PHYSIOL
SOMERVILLE C	1991	252	80	SCIENCE
THELEN J J	2002	400	245	ARCH BIOCHEM BIOPHYS
ZOU Z Y	2003	278	16414	J BIOL CHEM

L22 ANSWER 2 OF 46 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
AN 2004:832481 SCISEARCH
GA The Genuine Article (R) Number: 854KI
TI Increasing the carbon flux toward synthesis of short-**chain-length-medium-chain-length polyhydroxyalkanoate** in the peroxisome of Saccharomyces cerevisiae through modification of the beta-oxidation cycle
AU de Oliveira V C; Maeda I; Delessert S; Poirier Y (Reprint)
CS Univ Lausanne, Dept Biol Mol Vegetale, CH-1015 Lausanne, Switzerland (Reprint)
CYA Switzerland
SO APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (SEP 2004) Vol. 70, No. 9, pp. 5685-5687.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0099-2240.
DT Article; Journal
LA English
REC Reference Count: 15
AB Short-**chain-length-medium-chain-length polyhydroxyalkanoates** were synthesized in Saccharomyces cerevisiae from intermediates of the beta-oxidation cycle by expressing the **polyhydroxyalkanoate** synthases from Aeromonas caviae and Ralstonia eutropha in the peroxisomes. The quantity of polymer produced was increased by using a mutant of the beta-oxidation-associated multifunctional enzyme with low dehydrogenase activity toward R-3-hydroxybutyryl coenzyme A.
CC BIOTECHNOLOGY & APPLIED MICROBIOLOGY; MICROBIOLOGY
STP KeyWords Plus (R): MULTIFUNCTIONAL ENZYME; **TRANSGENIC** PLANTS; POLYESTERS; ACID
RE

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)
DOI Y	1995	28	4822	MACROMOLECULES
GIETZ D	1992	20	1425	NUCLEIC ACIDS RES
GLOVER J R	1994	269	7558	J BIOL CHEM
HAHN J J	1999	15	1053	BIOTECHNOL PROGR

MARCHESINI S .	2003	69	6495	APPL ENVIRON MICROB
MUMBERG 'D	1995	156	119	GENE
POIRIER Y	2001	71	209	ADV BIOCHEM ENG BIOT
POIRIER Y	1999	10	181	CURR OPIN BIOTECH
POIRIER Y	2002	207	97	FEMS MICROBIOL LETT
POIRIER Y	1995	13	142	BIO-TECHNOL
POIRIER Y	2001	67	5254	APPL ENVIRON MICROB
QIN Y M	1999	274	28619	J BIOL CHEM
REZZONICO E	2002	1	87	PHYTOCHEM REV
STEINBUCHEL A	1998	16	419	TRENDS BIOTECHNOL
SUDESH K	2000	25	1503	PROG POLYM SCI

L22 ANSWER 3 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 2003:995740 HCAPLUS
DN 140:164332
ED Entered STN: 23 Dec 2003
TI Chemical Synthesis of Crystalline Comb Polymers from Olefinic **Medium-Chain-Length** Poly[3-hydroxyalkanoates]
AU Hany, Roland; Boehlen, Christine; Geiger, Thomas; Hartmann, Rene; Kawada, Jumpei; Schmid, Manfred; Zinn, Manfred; Marchessault, Robert H.
CS Swiss Federal Laboratories for Materials Testing and Research (EMPA), Duebendorf, CH-8600, Switz.
SO Macromolecules (2004), 37(2), 385-389
CODEN: MAMOBX; ISSN: 0024-9297
PB American Chemical Society
DT Journal
LA English
CC 35-8 (Chemistry of Synthetic High Polymers)
AB Comb polymers were produced in a two-step synthesis from a bacterial poly[3-hydroxyalkanoate-co-3-hydroxyalkenoate] (PHOU, 1) containing 25 mol % terminal side-chain double bonds. The radical addition reaction of 11-mercaptoundecanoic acid to the side-chain alkenes of 1 produced derivative 2 containing thioether bonds with terminal carboxyl functionalities, which were subsequently **transformed** into the amide (3) or ester (4) derivs. using tridecylamine or octadecanol, resp. The reactions proceeded to completion with little side reactions, which was confirmed with NMR and GPC expts. The resulting comb polymers 3 and 4 were white crystalline materials. 13C CP/MAS NMR spectra and X-ray diffraction results suggested a crystalline textural two-phase organization into polyethylene-like domains and regions characteristic of poly[3-hydroxyalkanoates] (PHAs). The breadth of the decomposition steps in thermal gravimetric anal. and the diffuse melting endotherms confirmed the solid-state organization as composed of nanosize crystallites of both polyethylene and PHAs.
ST **polyhydroxyalkanoate** comb polymer
IT Melting point
(chemical synthesis of crystalline comb polymers from olefinic **medium-chain-length** poly[hydroxyalkanoates])
IT Polyesters, reactions
RL: RCT (Reactant); RACT (Reactant or reagent)
(chemical synthesis of crystalline comb polymers from olefinic **medium-chain-length** poly[hydroxyalkanoates])
IT 112-38-9, 10-Undecenoic acid 124-07-2, Octanoic acid, reactions
RL: RCT (Reactant); RACT (Reactant or reagent)
(chemical synthesis of crystalline comb polymers from olefinic **medium-chain-length** poly[hydroxyalkanoates])
IT 201933-09-7P
RL: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation); RACT (Reactant or reagent)
(chemical synthesis of crystalline comb polymers from olefinic **medium**

-chain-length poly[hydroxyalkanoates])
IT 112-92-5DP, 1-Octadecanol, reaction derivs. with carboxylic-side chain polyester 2869-34-3DP, 1-Aminotridecane, reaction derivs. with carboxylic-side chain polyester 71310-21-9DP, 11-Mercaptoundecanoic acid, reaction products with polyesters, derivs. 201933-09-7DP, reaction derivs.
RL: SPN (Synthetic preparation); PREP (Preparation)
(chemical synthesis of crystalline comb polymers from olefinic **medium**-**chain**-**length** poly[hydroxyalkanoates])
RE.CNT 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
(1) Abraham, G; Biomacromolecules 2001, V2, P562 HCAPLUS
(2) Bear, M; React Funct Polym 1997, V34, P65 HCAPLUS
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(15) Knorr, R; Tetrahedron Lett 1989, V30, P1927 HCAPLUS
(16) Koenig, J; Spectroscopy of Polymers; ACS Professional Reference, Chapter 9 1992
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(23) Neises, B; Angew Chem 1978, V90, P556 HCAPLUS
(24) Renard, E; Macromol Biosci 2003, V3, P248 HCAPLUS
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(26) Stigers, D; Biomacromolecules 2003, V4, P193 HCAPLUS
(27) Stranix, B; J Org Chem 1997, V62, P8987 HCAPLUS
(28) Zinn, M; Acta Biotechnol 2003, V23, P309 HCAPLUS

L22 ANSWER 4 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 1
AN 2004:951770 HCAPLUS
ED Entered STN: 10 Nov 2004
TI Recombinant bacterial system for producing mcl-**pha**
IN Lee, Sang Yeop; Park, Jong Pil; Park, Si Jae
PA Korea Advanced Institute of Science and Technology, S. Korea
SO Repub. Korean Kongkae Taeho Kongbo, No pp. given
CODEN: KRXXA7
DT Patent
LA Korean
IC ICM C12N015-70
CC 3 (Biochemical Genetics)
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	KR 2003070790	A	20030902	KR 2002-10325	20020226
PRAI	KR 2002-10325		20020226		

CLASS

PATENT NO.	CLASS	PATENT FAMILY CLASSIFICATION CODES
KR 2003070790	ICM	C12N015-70

AB PURPOSE: A recombinant bacterial system for producing MCL-**PHA** is provided, thereby mass-producing **medium-chain-length** poly(3-hydroxyalkanoate) (MCL-**PHA**).
CONSTITUTION: A recombinant expression vector containing **PHA** synthesizing gene phaC, gene fadF, and/or gene fadD is provided, wherein the recombinant vector is p10499613C2. A **transformant** **transformed** with the recombinant expression vector p10499613C2 is provided, wherein the **transformant** is Escherichia coli WA101(p10499613C2), Escherichia coli WB101(p10499613C2), Escherichia coli WAB101(p10499613C2), Escherichia coli KA101(p10499613C2), Escherichia coli KB101(p10499613C2) or Escherichia coli KAB101(p10499613C2). A method for producing MCL-**PHA** comprises culturing a **transformant** selected from Escherichia coli WA101(p10499613C2), Escherichia coli WB101(p10499613C2), Escherichia coli WAB101(p10499613C2), Escherichia coli KA101(p10499613C2), Escherichia coli KB101(p10499613C2) or Escherichia coli KAB101(p10499613C2) in a medium containing fatty acid.

L22 ANSWER 5 OF 46 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
AN 2003:949908 SCISEARCH
GA The Genuine Article (R) Number: 738GF
TI Effect of inactivation of poly(hydroxyalkanoates) depolymerase gene on the properties of poly(hydroxyalkanoates) in Pseudomonas resinovorans
AU Solaiman D K Y (Reprint); Ashby R D; Foglia T A
CS ARS, Eastern Reg Res Ctr, USDA, 600 E Marmaid Lane, Wyndmoor, PA 19038 USA (Reprint); ARS, Eastern Reg Res Ctr, USDA, Wyndmoor, PA 19038 USA
CYA USA
SO APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (OCT 2003) Vol. 62, No. 5-6, pp. 536-543.
Publisher: SPRINGER-VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010 USA.
ISSN: 0175-7598.
DT Article; Journal
LA English
REC Reference Count: 21
AB The phaZ gene of Pseudomonas resinovorans codes for a poly(hydroxyalkanoates) (**PHA**) depolymerase. Two phaZ mutants of Pseudomonas resinovorans NRRL B-2649, FOAC001 and FOAC002, were constructed by an in vitro transposition procedure followed by chromosomal integration via homologous recombination. A detailed mapping of the transposon insertion sites and an analysis of the resultant sequences showed that putative fusion polypeptides PhaZ(FOAC001) (239 amino-acid residues) and PhaZ(FOAC002) (85 amino-acid residues) could result from the mutant phaZ genes of FOAC001 and FOAC002, respectively. In vivo **PHA** degradation data indicated that PhaZ(FOAC001) might still retain a partial **PHA** depolymerization activity, while PhaZ(FOAC002) is completely devoid of this function. The cell yields and **PHA** contents of B-2649, FOAC001, and FOAC002 were similar when the cells were grown either under a limiting nitrogen-source (low-N) condition for up to 5 days or in excess N-source (high-N) for 3 days. A dramatic decrease in **PHA** content was observed in the PhaZ-active B-2649 and FOAC001 cells during prolonged cell growth (5 days) in high-N medium or in response to a shift-up in nitrogen-source. The repeat-unit compositions of the PHAs from FOAC001 and FOAC002 contained slightly lower amounts of beta-hydroxyoctanoate and higher beta-hydroxytetradecenoate than that of the wild-type B-2649 when grown under a high-N condition. While the molecular masses of the PHAs from FOAC001 and FOAC002 did not

vary under any conditions used in this study, those of the wild-type B-2649 were markedly increased in cells either grown for 5 days under a high-N condition or subjected to a nitrogen-source shift-up. These phaZ mutants thus provide a valuable system to study the influence of **PHA** depolymerase on the accumulation and properties of **medium-chain-length PHA.**

CC BIOTECHNOLOGY & APPLIED MICROBIOLOGY

STP KeyWords Plus (R): OLEOVORANS; **POLYHYDROXYALKANOATES**; DEGRADATION; METABOLISM; POLY(3-HYDROXYALKANOATES); IDENTIFICATION; **TRANSFORMATION**; INCLUSIONS; POLYESTERS; PROTEINS

RE

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)
ANDERSON A J	1990	54	450	MICROBIOL REV
ASHBY R D	2000	27	355	INT J BIOL MACROMOL
BRANDL H	1988	54	1977	APPL ENVIRON MICROB
CROMWICK A M	1996	46	464	APPL MICROBIOL BIOT
DESMET M J	1983	154	870	J BACTERIOL
DOI Y	1990	67	165	FEMS MICROBIOL LETT
FOSTER L J R	1994	118	279	FEMS MICROBIOL LETT
GORYSHIN I Y	1998	273	7367	J BIOL CHEM
HANAHAN D	1983	166	557	J MOL BIOL
HUISMAN G W	1992	38	1	APPL MICROBIOL BIOT
HUISMAN G W	1991	266	2191	J BIOL CHEM
JENDROSSEK D	2002	56	403	ANNU REV MICROBIOL
LAGEVEEN R G	1988	54	2924	APPL ENVIRON MICROB
POIRIER Y	1995	13	142	BIO-TECHNOL
REHM B H A	1999	25	3	INT J BIOL MACROMOL
RUIZ J A	2001	67	225	APPL ENVIRON MICROB
SAMBROOK J	1989			MOL CLONING LAB MANU
SOLAIMAN D K Y	1998	12	829	BIOTECHNOL TECH
SOLAIMAN D K Y	2002	24	245	BIOTECHNOL LETT
SOLAIMAN D K Y	2000	53	690	APPL MICROBIOL BIOT
STEINBUCHEL A	1991		123	BIOMATERIALS NOVEL M

L22 ANSWER 6 OF 46 MEDLINE on STN DUPLICATE 2

AN 2003315766 MEDLINE

DN PubMed ID: 12761653

TI Biosynthesis of **medium-chain-length** poly(hydroxyalkanoates) with altered composition by mutant hybrid **PHA** synthases.

AU Solaiman Daniel K Y

CS Eastern Regional Research Center, Agricultural Research Service, United States Department of Agriculture, Wyndmoor, PA 19038, USA.. dsolaiman@arserrc.gov

SO Journal of industrial microbiology & biotechnology, (2003 May) 30 (5) 322-6.
Journal code: 9705544. ISSN: 1367-5435.

CY England: United Kingdom

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 200311

ED Entered STN: 20030708
Last Updated on STN: 20031105
Entered Medline: 20031104

AB Pseudomonas resinovorans harbors two isogenic poly(hydroxyalkanoates) (PHAs) synthase genes (phaC1(Pre), phaC2(Pre)) responsible for the

production of intracellular **medium-chain-length** (mcl-)PHAs. Sequence analysis showed that the putative gene-products of these genes contain a conserved alpha/beta-hydrolase fold in the carboxy-terminal half of the proteins. Hybrid genes pha7 and pha8 were constructed by exchanging the alpha/beta-hydrolase-fold coding portions of phaC1(Pre) and phaC2(Pre) at the 3' terminal. When grown with decanoate as carbon source, the pha7- or pha8-**transformed** Escherichia coli LS1298 produced PHAs containing 73-75% beta-hydroxydecanoate (beta-HD) and 25-27% beta-hydroxyoctanoate (beta-HO). Deletion mutants, Delta pha7 and Delta pha8, were isolated during the PCR-based construction of pha7 and pha8, respectively. Cells harboring these mutants produced PHAs containing 55-60 mol% beta-HD and 40-45 mol% beta-HO. These results demonstrate the feasibility of generating active hybrid mcl-**PHA** synthase genes and their mutants with the potential of producing polymers having a varied repeat-unit composition.

CT Acyltransferases: CH, chemistry
*Acyltransferases: GE, genetics
Acyltransferases: IP, isolation & purification
*Acyltransferases: ME, metabolism
Amino Acid Sequence
Amino Acid Substitution: GE, genetics
*Decanoic Acids: ME, metabolism
Escherichia coli: EN, enzymology
Escherichia coli: GD, growth & development
Organisms, Genetically Modified: ME, metabolism
*Polyesters: ME, metabolism
Recombinant Fusion Proteins: CH, chemistry
Recombinant Fusion Proteins: IP, isolation & purification
Recombinant Fusion Proteins: ME, metabolism
Recombinant Fusion Proteins: UL, ultrastructure
*Sequence Deletion: GE, genetics

CN 0 (Decanoic Acids); 0 (Polyesters); 0 (Recombinant Fusion Proteins); EC 2.3. (Acyltransferases); EC 2.3.1.- (poly(3-hydroxyalkanoic acid) synthase)

L22 ANSWER 7 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 3
AN 2003:724822 HCAPLUS
DN 140:198131
ED Entered STN: 16 Sep 2003
TI Evidence of **medium-chain-length** polyhydroxyoctanoate accumulation in **transgenic** potato lines expressing the Pseudomonas oleovorans **Pha**-C1 polymerase in the cytoplasm
AU Romano, Andrea; Vreugdenhil, Dick; Jamar, Diaan; van der Plas, Linus H. W.; de Roo, Guy; Witholt, Bernard; Eggink, Gerrit; Mooibroek, Hans
CS Wageningen University and Research Centre, Wageningen, 6700 AA, Neth.
SO Biochemical Engineering Journal (2003), 16(2), 135-143
CODEN: BEJOFV; ISSN: 1369-703X
PB Elsevier Science B.V.
DT Journal
LA English
CC 16-3 (Fermentation and Bioindustrial Chemistry)
AB The phaC1 gene from Pseudomonas oleovorans, coding for the **Pha**-C1 polymerase, was introduced into the potato genome. **Transgenic** callus and plant lines which transcribed and translated the **transgene** were selected and cell suspension cultures from the wild type and **transgenic** lines were established. The substrate for the **Pha**-C1 polymerase, 3-(R)-hydroxyoctanoate, was provided to

the growth medium. In the **transgenic** lines, but not in the wild type or in **transgenic** cell suspension cultures without **Pha**-C1 expression, evidence of **medium-chain-length polyhydroxyalkanoate** accumulation ranging from 0.02 to 9.7 mg of polymer per g of dry weight was observed after feeding in the growth medium the substrate.

ST potato **transgenic PHA** synthase expression polyhydroxyoctanoate

IT Polyesters, preparation
RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)
(hydroxycarboxylic acid-based; polyhydroxyoctanoate accumulation in **transgenic** potato lines expressing the Pseudomonas oleovorans **Pha**-C1 polymerase)

IT Gene, microbial
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(phaC1; polyhydroxyoctanoate accumulation in **transgenic** potato lines expressing the Pseudomonas oleovorans **Pha**-C1 polymerase)

IT Genetic engineering
Transformation, genetic
(polyhydroxyoctanoate accumulation in **transgenic** potato lines expressing the Pseudomonas oleovorans **Pha**-C1 polymerase)

IT Plant tissue culture
(suspension; polyhydroxyoctanoate accumulation in **transgenic** potato lines expressing the Pseudomonas oleovorans **Pha**-C1 polymerase)

IT Solanum tuberosum
(**transgenic**; polyhydroxyoctanoate accumulation in **transgenic** potato lines expressing the Pseudomonas oleovorans **Pha**-C1 polymerase)

IT 44987-72-6, R-3-Hydroxyoctanoic acid
RL: BCP (Biochemical process); BIOL (Biological study); PROC (Process)
(polyhydroxyoctanoate accumulation in **transgenic** potato lines expressing the Pseudomonas oleovorans **Pha**-C1 polymerase)

IT 120659-38-3P
RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)
(polyhydroxyoctanoate accumulation in **transgenic** potato lines expressing the Pseudomonas oleovorans **Pha**-C1 polymerase)

IT 134688-88-3, **Pha** synthase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(polyhydroxyoctanoate accumulation in **transgenic** potato lines expressing the Pseudomonas oleovorans **Pha**-C1 polymerase)

RE.CNT 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD

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L22 ANSWER 8 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 4
AN 2002:391893 HCAPLUS
DN 136:396978
ED Entered STN: 24 May 2002
TI Production of **medium chain length polyhydroxyalkanoates** from fatty acid biosynthetic pathways
IN **Aquin, Stephanie; Peoples, Oliver P.; Snell, Kristi D.**
PA Metabolix, Inc., USA
SO PCT Int. Appl., 58 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM C12N015-82
CC 3-2 (Biochemical Genetics)
Section cross-reference(s): 7, 10, 11, 16
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002040690	A2	20020523	WO 2001-US43686	20011116
	WO 2002040690	A3	20030522		
	WO 2002040690	C2	20031120		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

CA	2428713	AA	20020523	CA 2001-2428713	20011116
AU	2002036464	A5	20020527	AU 2002-36464	20011116
US	2003017576	A1	20030123	US 2001-991152	20011116
EP	1334181	A2	20030813	EP 2001-985993	20011116
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
JP	2004534507	T2	20041118	JP 2002-543002	20011116
PRAI US	2000-249535P	P	20001117		
WO	2001-US43686	W	20011116		

CLASS

PATENT NO.	CLASS	PATENT FAMILY CLASSIFICATION CODES
WO 2002040690	ICM	C12N015-82
JP 2004534507	FTERM	2B030/AA02; 2B030/AA03; 2B030/AB04; 2B030/AD08; 2B030/CA17; 2B030/CB02; 2B030/CD07; 2B030/CD10; 4B024/AA03; 4B024/AA08; 4B024/BA07; 4B024/CA04; 4B024/DA01; 4B024/DA06; 4B024/EA04; 4B024/GA11; 4B063/QA01; 4B063/QA05; 4B063/QA18; 4B063/QQ32; 4B063/QR78; 4B063/QS38; 4B064/AD32; 4B064/CA02; 4B064/CA11; 4B064/CA19; 4B064/CC24; 4B064/DA11; 4B064/DA16; 4B065/AA26X; 4B065/AA41Y; 4B065/AA89X; 4B065/AB01; 4B065/AC14; 4B065/BA01; 4B065/BA02; 4B065/CA05; 4B065/CA10; 4B065/CA53

AB Methods for producing **polyhydroxyalkanoates** (PHAs) from fatty acid biosynthetic pathways using a 3-hydroxy acyl **ACP thioesterase**, a **PHA** synthase, and an acyl **CoA synthetase**, are disclosed. Methodol. for engineering plants to produce PHAs comprising **medium chain length** (D)-3-hydroxyacids from fatty acid biosynthetic pathways by expressing an enzyme having the catalytic activity of 3-hydroxyacyl **ACP thioesterase**, a **PHA** synthase capable of incorporating **medium chain** 3-hydroxyacids, and an enzyme having either (D)-3-**hydroxyacyl-CoA synthetase** activity or **CoA** transferase activity, has been developed. The methods described herein include expressing enzymes having 3-hydroxyacyl-**ACP thioesterase** activity in the plastids of leaves or seeds of plant crops or in an organism other than a plant such as bacteria in conjunction with, for example, an acyl **CoA synthetase** or **CoA** transferase, or a **PHA** synthase gene or genes, in the case of a two-subunit synthase, in the peroxisome, cytosol or plastids of higher plants. In some cases such as plastid expression of the **thioesterase** and **PHA** synthase, it is also useful to express a gene having a 3-**hydroxyacyl-CoA synthetase** activity in the plastid. Where the **PHA** synthase is expressed in the cytosol, it may optionally be useful to increase the expression of a gene or genes encoding an enzyme having the catalytic activity of a (D)-3-**hydroxyacyl-CoA synthetase**. Where the **PHA** synthase is targeted to the peroxisome, it may also be useful to also target an enzyme having the catalytic activity of a (D)-3-**hydroxyacyl-CoA synthetase** to the peroxisome. The methodol. described herein is useful for engineering both oil seed and biomass crops to produce the desired **PHA** biopolymers. Production of **medium chain length** PHAs in E. coli expressing PhaC, PhaG, and AlkK is described. Chloroplast-specific expression of PhaG and PhaC and

transformation of Arabidopsis thaliana is also described.

ST **medium chain polyhydroxyalkanoate** prodn fatty acid biosynthesis pathway; hydroxy acyl **ACP thioesterase polyhydroxyalkanoate** prodn; **PHA** synthase **polyhydroxyalkanoate** prodn acyl **CoA synthetase**

IT Gene, microbial
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(AlkK; production of **medium chain length polyhydroxyalkanoates** from fatty acid biosynthetic pathways)

IT Fatty acids, preparation
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(C8 and C10, increasing the levels of; production of **medium chain length polyhydroxyalkanoates** from fatty acid biosynthetic pathways)

IT Arabidopsis thaliana
Embryophyta
Escherichia coli
Eubacteria
Plant cell
Plant tissue
(**PHA** production in; production of **medium chain length polyhydroxyalkanoates** from fatty acid biosynthetic pathways)

IT Gene, microbial
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(PhaC; production of **medium chain length polyhydroxyalkanoates** from fatty acid biosynthetic pathways)

IT Gene, microbial
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(PhaG; production of **medium chain length polyhydroxyalkanoates** from fatty acid biosynthetic pathways)

IT Carboxylic acids, preparation
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(esters, polyhydroxy; production of **medium chain length polyhydroxyalkanoates** from fatty acid biosynthetic pathways)

IT Carboxylic acids, preparation
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(hydroxy, C8 and C10, increasing the levels of; production of **medium chain length polyhydroxyalkanoates** from fatty acid biosynthetic pathways)

IT Chloroplast
Leaf
Organelle
Peroxisome
Plastid
Seed
(**transgene** targeted to; production of **medium chain length polyhydroxyalkanoates** from fatty acid biosynthetic pathways)

IT Fats and Glyceridic oils, preparation
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP

(Preparation)
(vegetable, altering composition of; production of **medium chain length polyhydroxyalkanoates** from fatty acid biosynthetic pathways)

IT 9013-18-7P, 3-Hydroxyacyl-CoA synthase
RL: BPN (Biosynthetic preparation); CAT (Catalyst use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(3-hydroxyacyl CoA synthase, **medium chain length**; production of **medium chain length polyhydroxyalkanoates** from fatty acid biosynthetic pathways)

IT 68009-83-6P, 3-Hydroxyacyl-**ACP thioesterase**
RL: BPN (Biosynthetic preparation); CAT (Catalyst use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(3-hydroxyacyl-**ACP thioesterase**; production of **medium chain length polyhydroxyalkanoates** from fatty acid biosynthetic pathways)

IT 14292-26-3P, 3-Hydroxidecanoic acid 14292-27-4P, 3-Hydroxyoctanoic Acid 430430-82-3P, Butyl 3-hydroxyoctanote 430430-83-4P, Butyl 3-hydroxidecanoate
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(production of **medium chain length polyhydroxyalkanoates** from fatty acid biosynthetic pathways)

IT 134688-88-3P, **Polyhydroxyalkanoate** synthase 215314-08-2P, 3-Hydroxyacyl-ACP:coenzyme A acyltransferase
RL: BPN (Biosynthetic preparation); CAT (Catalyst use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(production of **medium chain length polyhydroxyalkanoates** from fatty acid biosynthetic pathways)

IT 429080-97-7 429080-98-8 429080-99-9 429081-00-5 429081-01-6 429081-02-7 429081-03-8 429081-04-9 429081-05-0, 9: PN: WO0240690 SEQID: 9 unclaimed DNA 429081-06-1 429081-07-2 429081-08-3 429081-09-4
RL: PRP (Properties)
(unclaimed nucleotide sequence; production of **medium chain length polyhydroxyalkanoates** from fatty acid biosynthetic pathways)

IT 80065-69-6 87733-53-7
RL: PRP (Properties)
(unclaimed sequence; production of **medium chain length polyhydroxyalkanoates** from fatty acid biosynthetic pathways)

L22 ANSWER 9 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 5
AN 2002:831840 HCAPLUS
DN 137:338415
ED Entered STN: 01 Nov 2002
TI Preparation of microbial **polyhydroxyalkanoates** and their use in toners
IN Honma, Tsutomo; Yano, Tetsuya; Nomoto, Tsuyoshi; Kozaki, Shinya
PA Canon Kabushiki Kaisha, Japan
SO Eur. Pat. Appl., 60 pp.
CODEN: EPXXDW
DT Patent
LA English
IC ICM C08G063-06
ICS C09D167-04; C09C003-10; C09B067-00; C12P007-62; G03G009-087; G03G007-00; B41M005-00

CC 35-5 (Chemistry of Synthetic High Polymers)
Section cross-reference(s): 74

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 1253160	A2	20021030	EP 2002-9695	20020429
	EP 1253160	A3	20031022		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
	JP 2003011312	A2	20030115	JP 2001-208704	20010710
	US 2003104302	A1	20030605	US 2002-133404	20020429
PRAI	JP 2001-131694	A	20010427		
	JP 2001-208704	A	20010710		

CLASS

PATENT NO.	CLASS	PATENT FAMILY CLASSIFICATION CODES
EP 1253160	ICM	C08G063-06
	ICS	C09D167-04; C09C003-10; C09B067-00; C12P007-62; G03G009-087; G03G007-00; B41M005-00
EP 1253160	ECLA	B41M005/00J10; C12P007/62A; G03G005/05C4B; G03G005/05C8; G03G005/05C6; G03G005/05C10; G03G009/087D4; G03G; G03G009/087H5; G03G009/087H3; C09D167/04
US 2003104302	ECLA	B41M005/00J10; G03G005/05C8; G03G005/05C4B; G03G005/05C10; G03G009/087D4; G03G009/087H3; G03G009/087H5; C09D167/04; C12P007/62A; G03G005/05C6

AB A construct comprises a base material and a **polyhydroxyalkanoate**, wherein at least a part of the base material is coated with the polyhyroxyalkanoate, and the **polyhydroxyalkanoate** comprises a 3-hydroxyalkanoic acid unit other than 3-hydroxypropionic acid unit, 3-hydroxy-n-butyric acid unit, and 3-hydroxy-n-valeric acid unit. A method for making a construct comprises: immobilizing a **medium chain length polyhydroxyalkanoate** synthetase to a base material, and reacting 3-hydroxyacyl CoA with the synthetase to synthesize a **polyhydroxyalkanoate** and to coat at least a part of the base material with the polyhdroxyalkanoate. **Polyhydroxyalkanoate** synthetase was isolated from a **transformant** having **polyhydroxyalkanoate** synthetase production capacity, immobilized on alumina, and incubated with (R)-3-hydroxyoctanoyl CoA to give a **polyhydroxyalkanoate.**

ST microbial **polyhydroxyalkanoate** manuf immobilized **polyhydroxyalkanoate** synthetase toner

IT Pseudomonas cichorii
(H45 and YN2, **polyhydroxyalkanoate** synthetase of; preparation of microbial **polyhydroxyalkanoates** and their use in toners)

IT Burkholderia
(OK3 and OK4, **polyhydroxyalkanoate** synthetase of; preparation of microbial **polyhydroxyalkanoates** and their use in toners)

IT Pseudomonas jessenii
(P161, **polyhydroxyalkanoate** synthetase of; preparation of microbial **polyhydroxyalkanoates** and their use in toners)

IT Pseudomonas putida
(P91, **polyhydroxyalkanoate** synthetase of; preparation of microbial **polyhydroxyalkanoates** and their use in toners)

IT Polyesters, preparation
RL: IMF (Industrial manufacture); PREP (Preparation)
(hydroxycarboxylic acid-based; preparation of microbial **polyhydroxyalkanoates** and their use in toners)

IT Burkholderia

Escherichia coli
Pseudomonas
(**polyhydroxyalkanoate** synthetase of; preparation of microbial **polyhydroxyalkanoates** and their use in toners)

IT Dyes
Electrophotographic toners
Pigments, nonbiological
(preparation of microbial **polyhydroxyalkanoates** and their use in toners)

IT 134688-88-3, **Polyhydroxyalkanoate** synthetase
RL: CAT (Catalyst use); USES (Uses)
(immobilized; preparation of microbial **polyhydroxyalkanoates** and their use in toners)

IT 147-14-8, Copper phthalocyanine
RL: TEM (Technical or engineered material use); USES (Uses)
(pigment; preparation of microbial **polyhydroxyalkanoates** and their use in toners)

IT 154994-48-6P 155075-32-4P 172923-04-5P 340255-66-5P 473988-23-7P
473988-24-8P 473988-26-0P 473988-28-2P 473988-29-3P 473988-30-6P,
Butyl acrylate-styrene-fumaric acid-propoxylated bisphenol A copolymer
RL: IMF (Industrial manufacture); TEM (Technical or engineered material use); PREP (Preparation); USES (Uses)
(preparation of microbial **polyhydroxyalkanoates** and their use in toners)

IT 85-61-0D, Coenzyme A, 3-hydroxyacyl
RL: RCT (Reactant); RACT (Reactant or reagent)
(preparation of microbial **polyhydroxyalkanoates** and their use in toners)

IT 1344-28-1, Alumina, uses
RL: TEM (Technical or engineered material use); USES (Uses)
(preparation of microbial **polyhydroxyalkanoates** and their use in toners)

IT 474039-52-6, 1: PN: EP1253160 SEQID: 1 unclaimed DNA 474039-53-7
474039-54-8 474042-99-4, 4: PN: EP1253160 SEQID: 4 unclaimed DNA
474043-00-0, 5: PN: EP1253160 SEQID: 5 unclaimed DNA 474043-01-1
474043-02-2 474043-03-3 474043-04-4 474043-05-5 474043-06-6
474043-07-7 474043-08-8
RL: PRP (Properties)
(unclaimed nucleotide sequence; preparation of microbial **polyhydroxyalkanoates** and their use in toners)

L22 ANSWER 10 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 6
AN 2002:796317 HCAPLUS
DN 138:135905
ED Entered STN: 20 Oct 2002
TI In vivo blending of **medium chain length** polyhydroxy-alkanoates and polyhydroxybutyrate using recombinant Pseudomonas putida harboring phbCAB operon of Ralstonia eutropha
AU Shin, Hyun-Dong; Lee, Jin-Nam; Lee, Yong-Hyun
CS College of Natural Sciences, Department of Genetic Engineering, Kyungpook National University, Taegu, 702-701, S. Korea
SO Biotechnology Letters (2002), 24(20), 1729-1735
CODEN: BILED3; ISSN: 0141-5492
PB Kluwer Academic Publishers
DT Journal
LA English
CC 16-4 (Fermentation and Bioindustrial Chemistry)
Section cross-reference(s): 3, 10, 35
AB The in vivo blending of **medium chain length**

polyhydroxyalkanoates (mcl-**PHA**) and polyhydroxybutyrate (PHB) was carried out using recombinant Pseudomonas putida after **transforming** the phbCAB operon of Ralstonia eutropha. The most suitable carbon sources for the production of mcl-**PHA** and PHB blends were identified to be octanoate and gluconate. The molar fractions of 3-hydroxyoctanoate and 3-hydroxybutyrate in the polymer blends were effectively modulated by controlling the mixing ratio of octanoate and gluconate, thereby producing a composition ranging from 95% mcl-**PHA** to 78% PHB.

ST Pseudomonas recombinant **polyhydroxyalkanoate**

IT Polyesters, preparation
RL: BPN (Biosynthetic preparation); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)
(hydroxycarboxylic acid-based; **medium chain length** polyhydroxy-alkanoate production by a recombinant Pseudomonas putida harboring the phbCAB operon)

IT Fermentation
Genetic engineering
Glass transition temperature
Melting point
Polydispersity
Pseudomonas putida
(**medium chain length** polyhydroxy-alkanoate production by a recombinant Pseudomonas putida harboring the phbCAB operon)

IT Operon
(phbCAB; **medium chain length** polyhydroxy-alkanoate production by a recombinant Pseudomonas putida harboring the phbCAB operon)

IT 50-99-7, Dextrose, processes 112-80-1, Oleic acid, processes 124-07-2, Octanoic acid, processes 334-48-5, Decanoic acid 526-95-4, Gluconic acid
RL: BCP (Biochemical process); BIOL (Biological study); PROC (Process)
(**medium chain length** polyhydroxy-alkanoate production by a recombinant Pseudomonas putida harboring the phbCAB operon)

IT 478912-37-7P 494221-27-1P 494221-28-2P 494221-29-3P 494221-30-6P 494221-31-7P
RL: BPN (Biosynthetic preparation); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)
(**medium chain length** polyhydroxy-alkanoate production by a recombinant Pseudomonas putida harboring the phbCAB operon)

IT 9028-41-5, Acetoacetylcoa reductase 9029-97-4, β-Ketothiolase 61461-50-5, Polyhydroxybutyrate synthase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**medium chain length** polyhydroxy-alkanoate production by a recombinant Pseudomonas putida harboring the phbCAB operon)

RE.CNT 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD

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L22 ANSWER 11 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 7
AN 2002:128703 HCAPLUS
DN 136:324133
ED Entered STN: 19 Feb 2002
TI Physiological Characterization and Genetic Engineering of Pseudomonas corrugata for **Medium-Chain-Length Polyhydroxyalkanoates** Synthesis from Triacylglycerols
AU Solaiman, Daniel K. Y.; Ashby, Richard D.; Foglia, Thomas A.
CS U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA, 19038, USA
SO Current Microbiology (2002), 44(3), 189-195
CODEN: CUMIDD; ISSN: 0343-8651
PB Springer-Verlag New York Inc.
DT Journal
LA English
CC 16-4 (Fermentation and Bioindustrial Chemistry)
Section cross-reference(s): 10
AB Pseudomonas belonging to the rRNA-DNA homol. group I produce **medium-chain-length** (mcl)-**polyhydroxyalkanoates** (**PHA**). We show that P. corrugata, a member of this group, accumulates 0.5-1.0 g of mcl-**PHA**/L of culture when grown on glucose (Gl) or oleic acid (Ol). The predominant monomers of Gl-**PHA** and Ol-**PHA** are β-hydroxydecanoate and β-hydroxyoctanoate, resp. The mol. masses and polydispersity of P. corrugata PHAs are higher than those typically found with other Pseudomonas. We electrotransformed P. corrugata with a plasmid pCN51lip-1 carrying Pseudomonas lipase genes to generate strain III111-1. The recombinant strain grew on intact triacylglycerols (TAGs) to 1.9-2.7 g of cell-dry-weight/L of culture. The yields and the predominant repeat-units of PHAs obtained from the lard- and tallow-grown III111-1 were similar to those of Ol-**PHA** from wild-type cells. In contrast to other Pseudomonas species, P. corrugata III111-1 grown on TAGs at temps. up to 36°C was not significantly affected with regard to cell yields, amts. of **PHA** produced, and the repeat unit compns. of the polymer.
ST Pseudomonas recombinant **polyhydroxyalkanoate** triacylglycerol
IT Electroporation
Genetic engineering
Pseudomonas corrugata
Temperature effects, biological
Transformation, genetic
(Pseudomonas corrugata **medium-chain-length polyhydroxyalkanoate** synthesis from triacylglycerols)
IT Coconut oil
Glycerides, processes

Lard
Soybean oil
Tallow
RL: BCP (Biochemical process); BIOL (Biological study); PROC (Process)
(Pseudomonas corrugata **medium-chain-length polyhydroxyalkanoate** synthesis from triacylglycerols)

IT Polyesters, preparation
RL: BPN (Biosynthetic preparation); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)
(hydroxycarboxylic acid-based; Pseudomonas corrugata **medium-chain-length polyhydroxyalkanoate** synthesis from triacylglycerols)

IT Gene, microbial
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(limA; Pseudomonas corrugata **medium-chain-length polyhydroxyalkanoate** synthesis from triacylglycerols)

IT Gene, microbial
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(lipA; Pseudomonas corrugata **medium-chain-length polyhydroxyalkanoate** synthesis from triacylglycerols)

IT 50-99-7, Dextrose, processes 112-80-1, Oleic acid, processes
RL: BCP (Biochemical process); BIOL (Biological study); PROC (Process)
(Pseudomonas corrugata **medium-chain-length polyhydroxyalkanoate** synthesis from triacylglycerols)

IT 389799-62-6P 415710-05-3P 415710-06-4P
RL: BPN (Biosynthetic preparation); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)
(Pseudomonas corrugata **medium-chain-length polyhydroxyalkanoate** synthesis from triacylglycerols)

IT 9001-62-1, Lipase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(Pseudomonas corrugata **medium-chain-length polyhydroxyalkanoate** synthesis from triacylglycerols)

RE.CNT 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD

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L22 ANSWER 12 OF 46 MEDLINE on STN DUPLICATE 8
AN 2002154632 MEDLINE
DN PubMed ID: 11886758
TI Synthesis of **polyhydroxyalkanoate** in the peroxisome of Pichia pastoris.
AU Poirier Yves; Erard Nadine; MacDonald-Comber Petetot Jean
CS Laboratoire de Biotechnologie Vegetale, Institut d'Ecologie, Universite de Lausanne, CH-1015 Lausanne, Switzerland.. yves.poirier@ie-bpv.unil.ch
SO FEMS microbiology letters, (2002 Jan 22) 207 (1) 97-102.
Journal code: 7705721. ISSN: 0378-1097.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200204
ED Entered STN: 20020312
Last Updated on STN: 20020501
Entered Medline: 20020430
AB **Polyhydroxyalkanoates** (PHAs) are polyesters naturally produced by bacteria that have properties of biodegradable plastics and elastomers. A **PHA** synthase from Pseudomonas aeruginosa modified at the carboxy-end for peroxisomal targeting was **transformed** in Pichia pastoris. The **PHA** synthase was expressed under the control of the promoter of the P. pastoris acyl-CoA oxidase gene. Synthesis of up to 1% **medium-chain-length PHA** per g dry weight was dependent on both the expression of the **PHA** synthase and the presence of oleic acid in the medium. **PHA** accumulated as inclusions within the peroxisomes. P. pastoris could be used as a model system to study how peroxisomal metabolism needs to be modified to increase **PHA** production in other eukaryotes, such as plants.
CT Check Tags: Support, Non-U.S. Gov't
Acyltransferases: GE, genetics
*Acyltransferases: ME, metabolism
Culture Media
Microscopy, Electron
*Peroxisomes: ME, metabolism
Peroxisomes: UL, ultrastructure
*Pichia: EN, enzymology
*Pichia: GE, genetics
*Polyesters: ME, metabolism
Pseudomonas aeruginosa: EN, enzymology
Pseudomonas aeruginosa: GE, genetics
Recombination, Genetic
CN 0 (Culture Media); 0 (Polyesters); EC 2.3. (Acyltransferases); EC 2.3.1.- (poly(3-hydroxyalkanoic acid) synthase)

L22 ANSWER 13 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 9
AN 2002:119111 HCAPLUS

DN 137:105556
ED Entered STN: 15 Feb 2002
TI Expression of a Gossypium hirsutum cDNA encoding a FatB palmitoyl-acyl carrier protein thioesterase in Escherichia coli
AU Huynh, Tu T.; Pirtle, Robert M.; Chapman, Kent D.
CS Department of Biological Sciences, Division of Biochemistry and Molecular Biology, University of North Texas, Denton, TX, 76203-5220, USA
SO Plant Physiology and Biochemistry (Paris, France) (2002), 40(1), 1-9
CODEN: PPBIEX; ISSN: 0981-9428
PB Editions Scientifiques et Medicales Elsevier
DT Journal
LA English
CC 7-2 (Enzymes)
Section cross-reference(s): 3, 6, 11
AB A cotton FatB cDNA encoding a palmitoyl-acyl carrier protein (**ACP**) **thioesterase** (Genbank Accession number AF034266) was expressed in various Escherichia coli strains. Transcription and translation in a coupled in vitro system revealed the presence of two [35S-Met]-labeled protein products, one of about 35 kDa and one of about 46 kDa. The 46 kDa polypeptide likely represented the translation of the preprotein while the 35 kDa polypeptide likely represented a translation product initiated at an alternative, internal in-frame initiation codon. Polyclonal anti-peptide antibodies were used to confirm the accumulation of this truncated protein. An immunoreactive 35 kDa protein was recognized in transformed E. coli cell lysates supporting the notion that indeed there was an internal start site, which seemed to be preferred when the cotton cDNA was expressed in E. coli. In crude homogenates of cotton embryos (30 dpa, days post anthesis) and cotyledons of 48 h dark-grown seedlings a 37 kDa protein, which likely represents the mature processed FatB protein, was recognized. When acyl-**CoA synthetase**-minus E. colimutants (K27 fadD88 mutant, CGSC 5478) were transformed with the cotton FatB cDNA, a four- to five-fold increase in C16:0 free fatty acid content was measured in the culture medium. Acyl-**ACP thioesterase** activity assays in E. coli lysates revealed that there was a clear preference for palmitoyl-ACP over oleoyl-ACP in vitro. Collectively, our results indicate that indeed the cotton FatB cDNA encodes a functional thioesterase with a preference for saturated acyl-ACPs (FatB) over unsatd. acyl-ACPs (FatA).
ST cloning cotton palmitoyl acyl carrier protein thioesterase
IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(ACP (acyl-carrier), conjugates with hexadecanoic acid; expression of Gossypium hirsutum cDNA encoding FatB palmitoyl-acyl carrier protein thioesterase in Escherichia coli)
IT Escherichia coli
Gossypium hirsutum
Molecular cloning
(expression of Gossypium hirsutum cDNA encoding FatB palmitoyl-acyl carrier protein thioesterase in Escherichia coli)
IT 57-10-3D, Hexadecanoic acid, conjugates with acyl carrier protein
68009-83-6, Hydrolase, acyl-[acyl carrier protein]
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(expression of Gossypium hirsutum cDNA encoding FatB palmitoyl-acyl carrier protein thioesterase in Escherichia coli)
RE.CNT 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
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L22 ANSWER 14 OF 46 WPIDS COPYRIGHT 2004 THE THOMSON CORP on STN
AN 2001-607194 [69] WPIDS
DNC C2001-180400
TI New DNA construct for the expression of multiple gene products including intein sequence(s) and herbicide resistance and desirable crop traits.
DC A23 C06 D16
IN SNELL, K D
PA (META-N) METABOLIX INC; (SNEL-I) SNELL K D
CYC 25
PI WO 2001059091 A2 20010816 (200169)* EN 30 C12N015-00
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
W: AU CA JP MX
AU 2001036839 A 20010820 (200175) C12N015-00
US 2002129400 A1 20020912 (200262) C12N015-82
EP 1255846 A2 20021113 (200282) EN C12N015-82
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR
ADT WO 2001059091 A2 WO 2001-US4254 20010209; AU 2001036839 A AU 2001-36839 20010209; US 2002129400 A1 Provisional US 2000-181739P 20000211, US 2001-779957 20010209; EP 1255846 A2 EP 2001-909045 20010209, WO 2001-US4254 20010209
FDT AU 2001036839 A Based on WO 2001059091; EP 1255846 A2 Based on WO 2001059091
PRAI US 2000-181739P 20000211; US 2001-779957 20010209
IC ICM C12N015-00; C12N015-82
ICS A01H005-00; C12N005-04; C12N015-63; C12P007-62; C12P007-64
AB WO 200159091 A UPAB: 20011126
NOVELTY - A DNA construct, C, for expression of multiple gene products comprising a first and second intein sequence where at least the first

intein sequence can catalyze excision of the exteins, is new.
DETAILED DESCRIPTION - The construct comprises:
(i) a single promoter at the 5' end of the construct;
(ii) multiple genes encoding one or more proteins;
(iii) a first intein sequence fused to the portion of the gene encoding the carboxy terminus of a first encoded protein;
(iv) a second intein sequence fused to the portion of the gene encoding the carboxy-terminus of a second encoded protein; and
(v) transcription termination sequences.
INDEPENDENT CLAIMS are included for the following:
(1) expression of multiple genes into cells comprising **transforming** the cells with C; and
(2) a cell producing recombinant proteins comprising C.
USE - The construct is useful to produce **transgenic** plants.
The traits conferred include herbicide and insect resistance and desirable plant crop traits (claimed).
Dwg.0/4
FS CPI
FA AB; DCN
MC CPI: A12-W04; A12-W11L; C04-A0800E; C04-E03E; C04-E03F; C04-E04; C04-F01; C04-F0800E; C14-B05; C14-V01; D05-H08; D05-H12A; D05-H12C; D05-H14B3; D05-H16B

L22 ANSWER 15 OF 46 MEDLINE on STN DUPLICATE 10
AN 2001571744 MEDLINE
DN PubMed ID: 11679353
TI Synthesis of **polyhydroxyalkanoate** in the peroxisome of Saccharomyces cerevisiae by using intermediates of fatty acid beta-oxidation.
AU Poirier Y; Erard N; Petetot J M
CS Laboratoire de Biotechnologie Vegetale, Institut d'Ecologie, Universite de Lausanne, CH-1015 Lausanne, Switzerland.. yves.poirier@ie-bpv.unil.ch
SO Applied and environmental microbiology, (2001 Nov) 67 (11) 5254-60. Journal code: 7605801. ISSN: 0099-2240.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200201
ED Entered STN: 20011029
Last Updated on STN: 20020125
Entered Medline: 20020117
AB **Medium-chain-length** **polyhydroxyalkanoates** (PHAs) are polyesters having properties of biodegradable thermoplastics and elastomers that are naturally produced by a variety of pseudomonads. Saccharomyces cerevisiae was **transformed** with the Pseudomonas aeruginosa PHAC1 synthase modified for peroxisome targeting by the addition of the carboxyl 34 amino acids from the Brassica napus isocitrate lyase. The PHAC1 gene was put under the control of the promoter of the catalase A gene. **PHA** synthase expression and **PHA** accumulation were found in recombinant S. cerevisiae growing in media containing fatty acids. **PHA** containing even-chain monomers from 6 to 14 carbons was found in recombinant yeast grown on oleic acid, while odd-chain monomers from 5 to 15 carbons were found in **PHA** from yeast grown on heptadecenoic acid. The maximum amount of **PHA** accumulated was 0.45% of the dry weight. Transmission electron microscopy of recombinant yeast grown on oleic acid revealed the presence of numerous **PHA** inclusions found within membrane-bound organelles. Together, these data

show that S. cerevisiae expressing a peroxisomal **PHA** synthase produces **PHA** in the peroxisome using the 3-hydroxyacyl coenzyme A intermediates of the beta-oxidation of fatty acids present in the media. S. cerevisiae can thus be used as a powerful model system to learn how fatty acid metabolism can be modified in order to synthesize high amounts of **PHA** in eukaryotes, including plants.

CT Check Tags: Support, Non-U.S. Gov't
*Acyltransferases: GE, genetics
*Acyltransferases: ME, metabolism
Blotting, Western
Fatty Acids: ME, metabolism
Inclusion Bodies: UL, ultrastructure
Microscopy, Electron
Oxidation-Reduction
*Peroxisomes: EN, enzymology
*Polyesters: ME, metabolism
Pseudomonas aeruginosa: EN, enzymology
Pseudomonas aeruginosa: GE, genetics
Recombination, Genetic
*Saccharomyces cerevisiae: EN, enzymology
Saccharomyces cerevisiae: GE, genetics
Saccharomyces cerevisiae: GD, growth & development
Saccharomyces cerevisiae: UL, ultrastructure

CN 0 (Fatty Acids); 0 (Polyesters); EC 2.3. (Acyltransferases); EC 2.3.1.- (poly(3-hydroxyalkanoic acid) synthase)

L22 ANSWER 16 OF 46 MEDLINE on STN DUPLICATE 11
AN 2001555115 MEDLINE
DN PubMed ID: 11601611
TI Production of **polyhydroxyalkanoates** from intact triacylglycerols by genetically engineered Pseudomonas.
AU Solaiman D K; Ashby R D; Foglia T A
CS US Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA 19038, USA.. dsolaiman@arserrc.gov
SO Applied microbiology and biotechnology, (2001 Sep) 56 (5-6) 664-9. Journal code: 8406612. ISSN: 0175-7598.
CY Germany: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200204
ED Entered STN: 20011017
Last Updated on STN: 20020419
Entered Medline: 20020418
AB Pseudomonas putida and P oleovorans have been extensively studied for their production of **medium-chain-length** (mcl)-**polyhydroxyalkanoates** (**PHA**). These bacteria are incapable of metabolizing triacylglycerols (TAGs). We have constructed recombinant P. putida and P. oleovorans that can utilize TAGs as substrates for growth and mcl-**PHA** synthesis. A recombinant plasmid, pCN51lip-1, carrying Pseudomonas lipase genes was used to electrotransform these organisms. The **transformants** expressed TAG-hydrolyzing activity as shown by a rhodamine B fluorescence plate assay. The genetically modified organisms grew in TAG-containing medium to a cell dry weight of 2-4 g/l. The recombinant P. putida produced mcl-**PHA** at a crude yield of 0.9-1.6 g/l with lard or coconut oil (Co) as substrate. While P. oleovorans **transformant** did not produce mcl-**PHA**, a mixed-culture fermentation approach with the wild-type and recombinant strains afforded polymer production from Co at a

crude yield of 0.5 g/l. Compositional analysis by gas chromatography/mass spectrometry showed that beta-hydroxyoctanoate (31-45 mol %) and beta-hydroxydecanoate (28-35 mol %) were the dominant repeat units of the TAG-based **PHA**. The number-average and weight-average molecular masses of the PHAs as determined by gel permeation chromatography were 82-170 x 10(3) g/mol and 464-693 x 10(3) g/mol, respectively. The recombinant approach can greatly increase the number of organisms that can be used to produce **PHA** from fat and oil substrates.

CT Culture Media
*Genetic Engineering: MT, methods
Lipase: GE, genetics
*Polyesters: ME, metabolism
*Pseudomonas: GE, genetics
Pseudomonas: GD, growth & development
*Pseudomonas: ME, metabolism
Pseudomonas putida: GE, genetics
Pseudomonas putida: GD, growth & development
Pseudomonas putida: ME, metabolism
Recombination, Genetic
*Triglycerides: ME, metabolism

CN 0 (Culture Media); 0 (Polyesters); 0 (Triglycerides); EC 3.1.1.3 (Lipase)

L22 ANSWER 17 OF 46 MEDLINE on STN DUPLICATE 12
AN 2001193568 MEDLINE
DN PubMed ID: 11217413
TI Production of polyesters in **transgenic** plants.
AU Poirier Y
CS Institut d'Ecologie-Biologie et Physiologie Vegetales, Universite de Lausanne, 1015 Lausanne, Switzerland.. yves.poirier@ie-bpv.unil.ch
SO Advances in biochemical engineering/biotechnology, (2001) 71 209-40. Ref: 98
Journal code: 8307733. ISSN: 0724-6145.
CY Germany: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 200104
ED Entered STN: 20010410
Last Updated on STN: 20010410
Entered Medline: 20010405

AB **Polyhydroxyalkanoates** (PHAs) are bacterial polyesters having the properties of biodegradable thermoplastics and elastomers. Synthesis of PHAs has been demonstrated in **transgenic** plants. Both polyhydroxybutyrate and the co-polymer poly(hydroxybutyrate-co-hydroxyvalerate) have been synthesized in the plastids of Arabidopsis thaliana and Brassica napus. Furthermore, a range of **medium-chain-length** PHAs has also been produced in plant peroxisomes. Development of agricultural crops to produce **PHA** on a large scale and at low cost will be a challenging task requiring a coordinated and stable expression of several genes. Novel extraction methods designed to maximize the use of harvested plants for **PHA**, oil, carbohydrate, and feed production will be needed. In addition to their use as plastics, PHAs can also be used to modify fiber properties in plants such as cotton. Furthermore, **PHA** can be exploited as a novel tool to study the carbon flux through various metabolic pathways, such as the fatty acid beta-oxidation cycle.

CT Arabidopsis: CH, chemistry

Arabidopsis: ME, metabolism
Brassica: CH, chemistry
Brassica: ME, metabolism
Chemical Engineering: MT, methods
Models, Biological
Oxygen: ME, metabolism
*Plants, Genetically Modified: CH, chemistry
*Plants, Genetically Modified: ME, metabolism
*Polyesters: CS, chemical synthesis
*Polyesters: CH, chemistry
Polyesters: IP, isolation & purification
Polyesters: ME, metabolism
RN 7782-44-7 (Oxygen)
CN 0 (Polyesters)

L22 ANSWER 18 OF 46 MEDLINE on STN DUPLICATE 13
AN 2001483968 MEDLINE
DN PubMed ID: 11330715
TI Heterologous expression of the acyl-acyl carrier protein thioesterase gene from the plant Umbellularia californica mediates polyhydroxyalkanoate biosynthesis in recombinant Escherichia coli.
AU Rehm B H; Steinbuchel A
CS Institut fur Mikrobiologie, Westfalische Wilhelms-Universitat Munster, Germany.. rehm@uni-muenster.de
SO Applied microbiology and biotechnology, (2001 Mar) 55 (2) 205-9.
Journal code: 8406612. ISSN: 0175-7598.
CY Germany: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200108
ED Entered STN: 20010903
Last Updated on STN: 20010903
Entered Medline: 20010830
AB The acyl-acyl carrier protein (**ACP**) **thioesterase** cDNA from the plant Umbellularia californica was functionally expressed in various recombinant Escherichia coli strains in order to establish a new metabolic route toward **medium-chain-length** polyhydroxyalkanoate (PHA(MCL)) biosynthesis from non-related carbon sources. Coexpression of the PHA synthase genes from Ralstonia eutropha and Pseudomonas aeruginosa, or only the PHA synthase gene from P. aeruginosa, respectively, showed PHA(MCL) accumulation when the type II PHA synthase from P. aeruginosa was produced. Both wild-type E. coli and various fad mutants were investigated; and only when the beta-oxidation pathway was impaired PHA(MCL) accumulation from gluconate was observed, contributing to about 6% of cellular dry weight. Thus coexpression of type II PHA synthase gene with cDNA encoding the **medium-chain** acyl-**ACP thioesterase** from U. californica established a new PHA(MCL) biosynthesis pathway, connecting fatty acid de novo biosynthesis with fatty acid beta-oxidation, using a non-related carbon source.
CT Acyltransferases: GE, genetics
*Acyltransferases: ME, metabolism
Escherichia coli: GE, genetics
*Escherichia coli: ME, metabolism
Fatty Acids: BI, biosynthesis
Genes, Plant
Gluconates: ME, metabolism
Lauraceae: EN, enzymology

*Lauraceae: GE, genetics
Oxidation-Reduction
Plasmids
*Polyesters: ME, metabolism
Pseudomonas aeruginosa: GE, genetics
Ralstonia eutropha: GE, genetics
Recombinant Proteins: ME, metabolism
*Thiolester Hydrolases: GE, genetics
*Thiolester Hydrolases: ME, metabolism

CN 0 (Fatty Acids); 0 (Gluconates); 0 (Plasmids); 0 (Polyesters); 0 (Recombinant Proteins); EC 2.3. (Acyltransferases); EC 2.3.1.- (poly(3-hydroxyalkanoic acid) synthase); EC 3.1.2. (Thiolester Hydrolases); EC 3.1.2.14 (oleoyl-(acyl-carrier-protein) hydrolase)

L22 ANSWER 19 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 14
AN 2000:911450 HCAPLUS
DN 134:67166
ED Entered STN: 29 Dec 2000
TI Multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**
IN Aquin, Stephanie; Peoples, Oliver P.; Snell, Kristi D.
PA Metabolix, Inc., USA
SO PCT Int. Appl., 57 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM C12N015-82
ICS C12N015-67; A01H005-00; A01K067-027
CC 3-2 (Biochemical Genetics)
Section cross-reference(s): 11, 16

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000078985	A1	20001228	WO 2000-US17197	20000623
	W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	CA 2375831	AA	20001228	CA 2000-2375831	20000623
	EP 1196614	A1	20020417	EP 2000-939991	20000623
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 2003503033	T2	20030128	JP 2001-505725	20000623
	AU 770120	B2	20040212	AU 2000-54991	20000623
PRAI	US 1999-140768P	P	19990624		
	WO 2000-US17197	W	20000623		

CLASS

PATENT NO.	CLASS	PATENT FAMILY CLASSIFICATION CODES
WO 2000078985	ICM	C12N015-82
	ICS	C12N015-67; A01H005-00; A01K067-027

AB Methods and constructs are provided for the introduction of multiple genes into plants using a single **transformation** event. Coordinated

expression of genes in the cassette, producing proteins with native amino acid sequences, is achieved by production of one polycistronic mRNA that contains sep. translation initiation signals for each enzyme coding region. Bicistronic constructs contain a single 5' promoter, protein encoding sequence 1, an IRES, protein encoding sequence 2, and a single 3' polyadenylation sequence. For polycistronic constructs, addnl. cassettes of protein encoding sequences, in which each coding region is preceded by an IRES, can be inserted between protein encoding sequence 2 and the polyadenylation sequence. The methods and constructs are useful for creating plants with stacked input traits (e.g., glyphosate tolerant plants producing BT toxin) and/or value added products (e.g., the production of **polyhydroxyalkanoates** (PHAs) in plants).

ST genetic vector plant multi gene **transformation** **polyhydroxyalkanoate** prodn

IT Albumins, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(2 S, tissue specific promoter from gene for; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Genetic element
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(IRES (internal ribosomal entry site) element, each **transgene** preceded by; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Cowpea mosaic virus
Potato virus Y
Turnip mosaic virus
(IRES from; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Reporter gene
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(as selectable marker; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT mRNA
RL: BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
(bicistronic; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Plant tissue
(callus, **transgenic**; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Transit peptides
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(chloroplast-, or peroxisome-targeting; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Alfalfa (Medicago sativa)
Pea

(chloroplast-targeting signal peptide of rubisco from; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Antibiotic resistance
(gene conferring, as selectable marker; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Chloroplast
Peroxisome
Plastid
(heterologous protein targeted to; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Polyesters, preparation
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(hydroxycarboxylic acid-based, **polyhydroxyalkanoates** (PHAs), **medium chain** and short **length**; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Drug delivery systems
(injections, micro-; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Drug delivery systems
(liposomes, **transformation** mediated by; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Plant tissue
(meristem, **transgenic**; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Breeding, plant
Electroporation
Genetic engineering
Genetic vectors
Microprojectile bombardment
Transformation, genetic
(multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT **Transgene**
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(multiple, expression construct containing; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Proteins, specific or class
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(oleosins, tissue specific promoter from gene for; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of

polyhydroxyalkanoates)

IT Pseudomonas aeruginosa
(phaC gene from; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Gene, microbial
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(phaC, from P. aeruginosa; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Gene, microbial
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(phaG; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Globulins, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(phaseolins, tissue specific promoter from gene for; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Promoter (genetic element)
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(plant, inducible, constitutive, and tissue specific; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Genetic element
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(polyadenylation signal; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT mRNA
RL: BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
(polycistronic; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Protoplast and Spheroplast
(polyethylene glycol-mediated **transformation** of; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Leaf
Seed
(promoter specific for expression in; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Ralstonia eutropha
(reductase and synthase genes from; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of

polyhydroxyalkanoates)

IT Herbicides
(resistance to, gene conferring, as selectable marker; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Proteins, specific or class
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(seed storage, tissue specific promoter from gene for; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Genetic element
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(signal sequence, chloroplast-targeting peptide encoding; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Translation initiation
(signals, separated for each protein; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Synthetic fibers
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(silicon, **transformation** mediated by; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Glutelins
Zeins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(tissue specific promoter from gene for; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Agrobacterium
(**transformation** mediated by; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Polyoxyalkylenes, biological studies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(**transformation** of protoplast mediated by; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Brassica
Coconut (Cocos nucifera)
Corn
Cottonseed
Flax
Mustard (Brassica)
Palm (Arecaceae)
Peanut (Arachis hypogaea)
Plant (Embryophyta)

Plant cell
Pollen
Safflower (Carthamus tinctorius)
Soybean (Glycine max)
Sunflower
(**transgenic**; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT Oxidation
(β-, enzyme complex for, α- and β-subunits for, **transgenes** for; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT 9027-23-0, Rubisco
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(chloroplast-targeting signal peptide of; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT 9037-80-3, Reductase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(gene for, from Alcaligenes entrophus; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT 134688-88-3, **Polyhydroxyalkanoate** synthase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(gene phaC for; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT 215314-08-2, 3-Hydroxyacyl-CoA ACP acyltransferase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(gene phaG for; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT 314102-59-5 314102-60-8 314102-61-9 314102-62-0 314102-63-1 314102-64-2 314102-65-3
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(nucleotide sequence; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT 9001-97-2, Starch branching enzyme 9030-10-8, Starch synthase 9031-55-4, Carboxylase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(tissue specific promoter from gene for; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT 25322-68-3, Polyethylene glycol
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(**transformation** of protoplast mediated by; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT 9001-05-2, Catalase 9029-97-4, β-Ketothiolase 61116-22-1, Acyl CoA oxidase

RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**transgene** for; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

IT 314103-65-6, 1: PN: WO0078985 SEQID: 1 unclaimed DNA 314103-66-7, 3: PN: WO0078985 SEQID: 2 unclaimed DNA 314103-67-8, 4: PN: WO0078985 SEQID: 3 unclaimed DNA 314103-68-9, 5: PN: WO0078985 SEQID: 4 unclaimed DNA 314103-69-0, 6: PN: WO0078985 SEQID: 5 unclaimed DNA 314103-70-3, 7: PN: WO0078985 SEQID: 6 unclaimed DNA
RL: PRP (Properties)
(unclaimed nucleotide sequence; multi-gene expression constructs for engineering plants with stacked input traits using a single **transformation** event and for production of **polyhydroxyalkanoates**)

RE.CNT 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

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(2) Korpela, T; WO 9854342 A 1998 HCAPLUS
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(4) Mittendorf, V; PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA 1998, V95(23), P13397 HCAPLUS
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(6) Sheen, J; EMBO (EUROPEAN MOLECULAR BIOLOGY ORGANIZATION) JOURNAL 1993, V12(9), P3497 HCAPLUS
(7) Skulachev, M; VIROLOGY 1999, V263(1), P139 HCAPLUS
(8) Univ Michigan; WO 9505472 A 1995 HCAPLUS

L22 ANSWER 20 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 15
AN 2000:742235 HCAPLUS
DN 133:291952
ED Entered STN: 20 Oct 2000
TI Modification of lipid biosynthesis by DNA shuffling
IN Yuan, Ling; Raillard, Sun Ai; Lassner, Michael
PA Maxygen, Inc., USA
SO PCT Int. Appl., 90 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM C12N015-10
ICS C12N015-82; A01H005-00
CC 3-2 (Biochemical Genetics)
Section cross-reference(s): 7, 11

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000061740	A1	20001019	WO 2000-US9285	20000406

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRAI US 1999-128707P P 19990410
CLASS

PATENT NO.	CLASS	PATENT FAMILY CLASSIFICATION CODES
WO 2000061740	ICM	C12N015-10
	ICS	C12N015-82; A01H005-00

AB Methods of modulating lipid production in cells and whole organisms by DNA shuffling are provided. Single genes, operons, lipid biosynthetic cycles and whole genomes can be recombined to produce cells and organisms with desirable lipid synthetic or metabolic activity. Libraries of recombined lipid synthetic nucleic acids and organisms are also provided. Modification of lipid saturation, fatty acid composition, fatty alc. composition, wax composition, acyl chain length, location of fatty acid accumulation, triglyceride yield, substrate specificity, expression level, are described. A decrease in susceptibility to protease cleavage, high or low pH levels, extreme temps., are also claimed. A decrease in toxicity, and modification of methyltransferase activity resulting in formation of branched chain, cyclopropyl, methoxy, or keto fatty acids, are also described. Use of two-hybrid system in detecting the changes in lipid biosynthetic activity is also claimed. Screening of libraries, such as phage display library is described. Crop plants such as corn, peanut, barley, millet, rice, soybean, sorghum, wheat, oats, sunflower, or nut whose lipid biosynthetic activity modified, are claimed. DNA shuffling is a powerful process for directed evolution, which generates diversity by recombination, combining useful mutations from individual genes.

ST lipid biosynthesis modification plant DNA shuffling

IT Proteins, specific or class
RL: BPN (Biosynthetic preparation); CAT (Catalyst use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(ACP (acyl-carrier), 3-hydroxy acyl; modification of lipid biosynthesis by DNA shuffling)

IT Proteins, specific or class
RL: BPN (Biosynthetic preparation); CAT (Catalyst use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(ACP (acyl-carrier); modification of lipid biosynthesis by DNA shuffling)

IT Proteins, specific or class
RL: BPN (Biosynthetic preparation); CAT (Catalyst use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(DNA-binding; modification of lipid biosynthesis by DNA shuffling)

IT Proteins, specific or class
RL: BPN (Biosynthetic preparation); CAT (Catalyst use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(FABP (fatty acid-binding protein); modification of lipid biosynthesis by DNA shuffling)

IT Genetic element
RL: BPN (Biosynthetic preparation); CAT (Catalyst use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(Lox, protein; modification of lipid biosynthesis by DNA shuffling)

IT Operon
(PKS-like; modification of lipid biosynthesis by DNA shuffling)

IT Fatty acids, biological studies
Waxes
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PREP (Preparation)
(composition, modification of; modification of lipid biosynthesis by DNA shuffling)

IT Protein degradation
(decrease in susceptibility to; modification of lipid biosynthesis by

DNA shuffling)
IT Cytotoxicity
(decrease in; modification of lipid biosynthesis by DNA shuffling)
IT Alcohols, biological studies
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PREP (Preparation)
(fatty, composition, modification of; modification of lipid biosynthesis by DNA shuffling)
IT Recombination, genetic
(gene shuffling; modification of lipid biosynthesis by DNA shuffling)
IT pH
(high or low, stability against; modification of lipid biosynthesis by DNA shuffling)
IT Cyanobacteria
Escherichia coli
Pseudomonas putida
Synechocystis
(library; modification of lipid biosynthesis by DNA shuffling)
IT Operon
(lux; modification of lipid biosynthesis by DNA shuffling)
IT Algae
Animal
Bacteria (Eubacteria)
Fungi
Genetic engineering
Phage display library
Plant (Embryophyta)
Thermal stability
(modification of lipid biosynthesis by DNA shuffling)
IT Lipids, biological studies
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PREP (Preparation)
(modification of lipid biosynthesis by DNA shuffling)
IT Proteins, specific or class
RL: BPN (Biosynthetic preparation); CAT (Catalyst use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(oleosins; modification of lipid biosynthesis by DNA shuffling)
IT Proteins, specific or class
RL: BPN (Biosynthetic preparation); CAT (Catalyst use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(phospholipid-exchanging, phosphatidylcholine; modification of lipid biosynthesis by DNA shuffling)
IT Proteins, specific or class
RL: BPN (Biosynthetic preparation); CAT (Catalyst use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(sulfolipid biosynthesis; modification of lipid biosynthesis by DNA shuffling)
IT Barley
Compositae (Asteraceae)
Corn
Crop (plant)
Grass (Poaceae)
Legume (Fabaceae)
Millet
Oat
Peanut (Arachis hypogaea)
Rice (Oryza sativa)

Sorghum
Soybean (Glycine max)
Sunflower
Wheat
(transgenic; modification of lipid biosynthesis by DNA shuffling)
IT Genetic methods
(two-hybrid screening; modification of lipid biosynthesis by DNA shuffling)
IT Fatty acids, biological studies
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PREP (Preparation)
(unsatd.; modification of lipid biosynthesis by DNA shuffling)
IT Glycerides, biological studies
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PREP (Preparation)
(yield; modification of lipid biosynthesis by DNA shuffling)
IT Oxidation
(β-, enzyme for; modification of lipid biosynthesis by DNA shuffling)
IT 9067-83-8P, CDP-diacylglycerol synthase
RL: BPN (Biosynthetic preparation); CAT (Catalyst use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(ER; modification of lipid biosynthesis by DNA shuffling)
IT 9025-77-8P, Phosphatidic acid phosphatase 9033-46-9P, Phosphatidylglycerol phosphatase 9068-49-9P, Phosphatidylglycerophosphate synthase 9082-66-0P, Linoleate desaturase 72536-70-0P, Oleate desaturase
RL: BPN (Biosynthetic preparation); CAT (Catalyst use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(Plastidial and ER; modification of lipid biosynthesis by DNA shuffling)
IT 9001-62-1P, Lipase 9001-86-9P, Phospholipase C 9001-87-0P, Phospholipase D 9013-18-7P, Long-chain acyl-**CoA synthetase** 9023-93-2P, Acetyl CoA carboxylase 9026-13-5P, Diacylglycerol choline phosphotransferase 9026-34-0P, Cholinephosphate cytidylyltransferase 9026-67-9P, Choline kinase 9027-01-4P 9028-40-4P, β-Ketoacyl reductase 9029-60-1P, Lipoxygenase 9029-96-3P, Glycerol-3-phosphate acyltransferase 9031-56-5P, Ligase 9033-25-4P, Methyltransferase 9037-80-3P, Reductase 9054-78-8P, Phosphatidylserine decarboxylase 9077-10-5P, β-Ketoacyl-ACP synthase 37250-34-3P, β-Ketoacyl-ACP reductase 37251-08-4P, Enoyl-ACP reductase 37256-86-3P, Stearoyl-ACP desaturase 37257-17-3P, Malonyl-CoA transacylase 37277-55-7P, Monogalactosyldiacyl-glycerol synthase 51845-48-8P, Cyclopropane fatty acid synthase 51901-16-7P 58943-36-5P, Thioesterase 60382-71-0P, Diacylglycerol kinase 68009-83-6P, Acyl-**ACP thioesterase** 69403-06-1P, Fatty acid Elongase 69913-00-4P, UDP-galactose:diacylgalactosylglycerol galactosyltransferase 71833-11-9P, Hydroperoxide lyase 77322-37-3P, Acyl-acyl carrier protein synthase 88414-92-0P 94219-29-1P, Fatty acid Elongase 103843-28-3P, Desaturase 115926-52-8P, Phosphatidylinositol-3-kinase 159202-88-7P, Cis-trans-Fatty acid isomerase 300669-15-2P, Palmitoylphosphatidylglycerol desaturase 300676-64-6P, Monogalactosyldiacylglycerol palmitoyl-specific desaturase
RL: BPN (Biosynthetic preparation); CAT (Catalyst use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(modification of lipid biosynthesis by DNA shuffling)
RE.CNT 12 THERE ARE 12 CITED REFERENCES AVAILABLE FOR THIS RECORD

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L22 ANSWER 21 OF 46 WPIDS COPYRIGHT 2004 THE THOMSON CORP on STN
AN 2000-664817 [64] WPIDS
DNN N2000-492753 DNC C2000-201293
TI Producing **polyhydroxyalkanoate** (**PHA**) in recombinant organism comprising DNA sequence encoding dehydrogenase enzyme involves providing ketoacid-CoA to yield hydroxy-acyl-CoA, that serves a substrate for **PHA** synthase.
DC A23 C06 D16 P13
IN **AQUIN, S**; VEZINA, L
PA (MIAC) CANADA MIN AGRIC & AGRI-FOOD CANADA; (UYLA-N) UNIV LAVAL; (MIAC) CANADA DEPT AGRIC & AGRI-FOOD CANADA
CYC 91
PI WO 2000055328 A1 20000921 (200064)* EN 56 C12N015-52
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
AU 2000032668 A 20001004 (200101) C12N015-52
EP 1161539 A1 20011212 (200204) EN C12N015-52
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
US 6492134 B1 20021210 (200301) C12P001-00
ADT WO 2000055328 A1 WO 2000-CA275 20000315; AU 2000032668 A AU 2000-32668 20000315; EP 1161539 A1 EP 2000-910451 20000315, WO 2000-CA275 20000315; US 6492134 B1 Provisional US 1999-124417P 19990315, US 2000-526098 20000315
FDT AU 2000032668 A Based on WO 2000055328; EP 1161539 A1 Based on WO 2000055328
PRAI US 1999-124417P 19990315; US 2000-526098 20000315
IC ICM C12N015-52; C12P001-00
ICS A01H005-00; C07H021-02; C12N015-53; C12N015-54; C12N015-55; C12N015-82; C12N015-87
AB WO 200055328 A UPAB: 20011206
NOVELTY - Producing **polyhydroxyalkanoate** (**PHA**) comprising selecting a **transgenic** organism comprising a foreign DNA sequence (I) encoding an enzyme having dehydrogenase activity, providing a keto acid-CoA which will yield the **PHA** substrate R-(-)-hydroxyacyl-CoA when acted upon by the enzyme, and producing **PHA**, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) a cloning vector (II) comprising (I) which encodes an enzyme

having dehydrogenase activity that will produce R-(-)-hydroxyacyl-CoA from a keto acid-CoA;
(2) a host cell (III) comprising (I);
(3) a **transgenic** organism (IV) comprising (I); and
(4) producing a **polyhydroxyalkanoate** in a host, comprising:
(a) selecting a host for expression of genes encoding enzymes needed for **PHA** synthesis;
(b) introducing into the host structural genes encoding a **thioesterase**, an acyl-CoA **synthetase**, a thiolase, a hydroyacyl-CoA dehydrogenase, and a **PHA** synthase;
(c) expressing the genes encoding the enzymes; and
(d) providing substrates for the enzyme to produce **PHA**.
USE - For producing **polyhydroxyalkanoates** in **transgenic** plants (claimed).
ADVANTAGE - Through the engineering of the new metabolic pathway, R-(-)-3-OH-acyl-CoAs monomer subunits of adequate length are produced which serve as substrate for activity of the **PHA** synthases.
DESCRIPTION OF DRAWING(S) - The figure shows the synthetic pathway involved in the production of **medium chain length polyhydroxyalkanoates**.
Dwg.3/6
FS CPI GMPI
FA AB; GI; DCN
MC CPI: A02-A; A03-C02; A05-E02; A10-A; A10-D05; C04-E08; C04-F0100E; C04-P0100E; C10-E04C; D05-H12E; D05-H14; D05-H16B

L22 ANSWER 22 OF 46 MEDLINE on STN DUPLICATE 16
AN 2000245561 MEDLINE
DN PubMed ID: 10781572
TI FabG, an NADPH-dependent 3-ketoacyl reductase of Pseudomonas aeruginosa, provides precursors for **medium-chain-length** poly-3-hydroxyalkanoate biosynthesis in Escherichia coli.
AU Ren Q; Sierro N; Witholt B; Kessler B
CS Institute of Biotechnology, ETH Honggerberg, CH-8093 Zurich, Switzerland.
SO Journal of bacteriology, (2000 May) 182 (10) 2978-81.
Journal code: 2985120R. ISSN: 0021-9193.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200006
ED Entered STN: 20000622
Last Updated on STN: 20000622
Entered Medline: 20000612
AB Escherichia coli hosts expressing fabG of Pseudomonas aeruginosa showed 3-ketoacyl coenzyme A (CoA) reductase activity toward R-3-hydroxyoctanoyl-CoA. Furthermore, E. coli recombinants carrying the poly-3-hydroxyalkanoate (**PHA**) polymerase-encoding gene phaC in addition to fabG accumulated **medium-chain-length** PHAs (mcl-PHAs) from alkanoates. When E. coli fadB or fadA mutants, which are deficient in steps downstream or upstream of the 3-ketoacyl-CoA formation step during beta-oxidation, respectively, were **transformed** with fabG, higher levels of **PHA** were synthesized in E. coli fadA, whereas similar levels of **PHA** were found in E. coli fadB, compared with those of the corresponding mutants carrying phaC alone. These results strongly suggest that FabG of P. aeruginosa is able to reduce mcl-3-ketoacyl-CoAs generated by the beta-oxidation to 3-hydroxyacyl-CoAs to provide precursors for the **PHA** polymerase.
CT Check Tags: Support, Non-U.S. Gov't

3-Hydroxyacyl CoA Dehydrogenases: GE, genetics
*3-Hydroxyacyl CoA Dehydrogenases: ME, metabolism
Acyltransferases: GE, genetics
Alcohol Oxidoreductases: GE, genetics
Cloning, Molecular
Escherichia coli: GE, genetics
*Escherichia coli: ME, metabolism
Genes, Bacterial
Hydroxy Acids: ME, metabolism
*Pseudomonas aeruginosa: EN, enzymology
Recombination, Genetic

CN 0 (Hydroxy Acids); EC 1.1 (Alcohol Oxidoreductases); EC 1.1.1.35 (3-Hydroxyacyl CoA Dehydrogenases); EC 1.1.1.36 (acetoacetyl-CoA reductase); EC 2.3. (Acyltransferases); EC 2.3.1.- (poly-beta-hydroxybutyrate polymerase)

L22 ANSWER 23 OF 46 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN DUPLICATE 17

AN 2000:360335 BIOSIS

DN PREV200000360335

TI Characterisation of enzymes determining fatty acid **chain length** in developing seeds of Limnanthes douglasii.

AU Sandager, Line [Reprint author]; Stymne, Sten

CS Department of Medicinal Chemistry, Royal Danish School of Pharmacy, Universitetsparken 2, DK-2100, Copenhagen, Denmark

SO Journal of Plant Physiology, (May, 2000) Vol. 156, No. 5-6, pp. 617-622. print.
CODEN: JPPHEY. ISSN: 0176-1617.

DT Article

LA English

ED Entered STN: 23 Aug 2000
Last Updated on STN: 8 Jan 2002

AB Total fatty acid composition was determined in Limnanthes douglasii seeds during different stages of development and acyl specificities of acyl-**ACP thioesterase** and acyl-CoA elongase activities were investigated in extracts of the developing seeds. It was concluded that L. douglasii developing seeds possess acyl-**ACP thioesterase**(s) with high activity towards saturated acyl-ACPs of a **chain length** of C14-C18 as well as oleoyl (18 : 1DELTA9)-ACP. L. douglasii acyl-CoA elongase(s) efficiently elongated 14 : 0-CoA to 20 : 0 fatty acids but not to 22 : 0 whereas 18 : 1DELTA9-CoA was efficiently elongated to 22 : 1DELTA13 (erucic acid). The results obtained suggest that 20 : 1DELTA5, the dominating very long chain fatty acid in L. douglasii seeds, is synthesised from mainly 14 : 0 fatty acids produced in the plastid, which are elongated to 20 : 0-CoA in the cytosolic compartment and then further desaturated at the DELTA5 position. The erucic acid present in L. douglasii is synthesised from oleic acid exported from the plastid, which is elongated to 22 carbons by an acyl-CoA elongase enzyme that appears distinct from that responsible for the production of 20 : 0.

CC Enzymes - General and comparative studies: coenzymes 10802

IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics)

IT Chemicals & Biochemicals
fatty acid **chain**: **length**; fatty acid elongase; **medium chain** thioesterase

IT Miscellaneous Descriptors
seed development

ORGN Classifier

Limnanthaceae 26280
Super Taxa
Dicotyledones; Angiospermae; Spermatophyta; Plantae
Organism Name
Limnanthes douglasii [meadowfoam]: seed
Taxa Notes
Angiosperms, Dicots, Plants, Spermatophytes, Vascular Plants
RN 69403-06-1Q (fatty acid elongase)
94219-29-1Q (fatty acid elongase)
9013-18-7Q (FATTY ACID ELONGASE)
9080-51-7Q (FATTY ACID ELONGASE)

L22 ANSWER 24 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 2000:155714 HCAPLUS
DN 133:54269
ED Entered STN: 09 Mar 2000
TI Molecular cloning of two (R)-specific enoyl-CoA hydratase genes from Pseudomonas aeruginosa and their use for **polyhydroxyalkanoate** synthesis
AU Tsuge, T.; Fukui, T.; Matsusaki, H.; Taguchi, S.; Kobayashi, G.; Ishizaki, A.; Doi, Y.
CS Graduate School of Bioresource and Bioenvironmental Science, Division of Bioscience and Biotechnology, Kyushu University, Higashi-ku, Fukuoka, Japan
SO FEMS Microbiology Letters (2000), 184(2), 193-198
CODEN: FMLED7; ISSN: 0378-1097
PB Elsevier Science B.V.
DT Journal
LA English
CC 3-2 (Biochemical Genetics)
Section cross-reference(s): 7, 10, 16
AB Two Pseudomonas aeruginosa genes, termed phaJ1Pa and phaJ2Pa, homologous to the Aeromonas caviae (R)-specific enoyl-CoA hydratase gene (phaJAc) were cloned using a PCR technique to investigate the monomer-supplying ability for **polyhydroxyalkanoate** (**PHA**) synthesis from β-oxidation cycle. Two expression plasmids for phaJ1Pa and phaJ2Pa were constructed and introduced into Escherichia coli DH5α strain. The recombinants harboring phaJ1Pa or phaJ2Pa showed high (R)-specific enoyl-CoA hydratase activity with different substrate specificities, i.e., specific for short chain-**length** enoyl-CoA or **medium chain-length** enoyl-CoA, resp. In addition, co-expression of these two hydratase genes with **PHA** synthase gene in E. coli LS5218 resulted in the accumulation of **PHA** up to 14-29 wt% of cell dry weight from dodecanoate as a sole carbon source. It has been suggested that phaJ1Pa and phaJ2Pa products have the monomer-supplying ability for **PHA** synthesis from β-oxidation cycle.
ST DNA sequence Pseudomonas gene phaJ1 phaJ2 enoyl CoA hydratase; recombinant prodn Pseudomonas gene phaJ1 phaJ2 enoyl CoA hydratase; **polyhydroxyalkanoate** synthesis **transformed** Escherichia Pseudomonas phaJ1 enoyl CoA hydratase; **transformed** Escherichia **polyhydroxyalkanoate** synthesis Pseudomonas phaJ2 enoyl CoA hydratase
IT Pseudomonas aeruginosa
(Pseudomonas aeruginosa gene phaJ1Pa and phaJ2Pa (R)-specific enoyl-CoA hydratases, sequences, recombinant production, and substrate specificities thereof)
IT Carboxylic acids, biological studies
RL: BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)

(hydroxy; **polyhydroxyalkanoate** (**PHA**) accumulation in Escherichia coli **transformed** with expression vectors pEH6 (contains P. aeruginosa gene phaJ1Pa) and pBE14 (contains P. aeruginosa gene phaJ2Pa))

IT Molecular cloning
(mol. cloning of two (R)-specific enoyl-CoA hydratase genes (phaJ1Pa and phaJ2Pa) from Pseudomonas aeruginosa and their use in **polyhydroxyalkanoate** synthesis)

IT Protein sequences
(of Pseudomonas aeruginosa gene phaJ1Pa and phaJ2Pa (R)-specific enoyl-CoA hydratases)

IT DNA sequences
(of Pseudomonas aeruginosa genes phaJ1Pa and phaJ2Pa, encode (R)-specific enoyl-CoA hydratases)

IT Plasmid vectors
(pEH6 and pBE14; mol. cloning of two (R)-specific enoyl-CoA hydratase genes (phaJ1Pa and phaJ2Pa) from Pseudomonas aeruginosa and their use in **polyhydroxyalkanoate** synthesis)

IT Gene, microbial
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(phaJ1Pa and phaJ2Pa; mol. cloning of two (R)-specific enoyl-CoA hydratase genes (phaJ1Pa and phaJ2Pa) from Pseudomonas aeruginosa and their use in **polyhydroxyalkanoate** synthesis)

IT Escherichia coli
(**polyhydroxyalkanoate** (**PHA**) accumulation in Escherichia coli **transformed** with expression vectors pEH6 (contains P. aeruginosa gene phaJ1Pa) and pBE14 (contains P. aeruginosa gene phaJ2Pa))

IT 276269-66-0P 276269-67-1P
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation)
(amino acid sequence; Pseudomonas aeruginosa gene phaJ1Pa and phaJ2Pa (R)-specific enoyl-CoA hydratases, sequences, recombinant production, and substrate specificities thereof)

IT 9027-13-8P, Short chain enoyl-CoA hydratase
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation)
(gene phaJ1Pa; Pseudomonas aeruginosa gene phaJ1Pa and phaJ2Pa (R)-specific enoyl-CoA hydratases, sequences, recombinant production, and substrate specificities thereof)

IT 168680-23-7P, Medium-chain 2-enoyl-CoA hydratase
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation)
(gene phaJ2Pa; Pseudomonas aeruginosa gene phaJ1Pa and phaJ2Pa (R)-specific enoyl-CoA hydratases, sequences, recombinant production, and substrate specificities thereof)

IT 259517-21-0, GenBank AB040025 259517-22-1, GenBank AB040026
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(nucleotide sequence; DNA sequence and mol. cloning of two (R)-specific enoyl-CoA hydratase genes (phaJ1Pa and phaJ2Pa) from Pseudomonas aeruginosa and their use in **polyhydroxyalkanoate** synthesis)

IT 10191-24-9D, Hexanoic acid, 3-hydroxy-, copolymer with 3-hydroxyoctanoic acid 14292-27-4D, 3-Hydroxyoctanoic acid, copolymer with 3-hydroxyhexanoic acid 147398-31-0, 3-Hydroxybutyric

acid-3-hydroxyhexanoic acid copolymer
RE: BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
(**polyhydroxyalkanoate** (**PHA**) accumulation in Escherichia coli **transformed** with expression vectors pEH6 (contains P. aeruginosa gene phaJ1Pa) and pBE14 (contains P. aeruginosa gene phaJ2Pa))

RE.CNT 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

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(16) Ren, Q; Appl Microbiol Biotechnol 1998, V49, P743 HCAPLUS
(17) Sambrook, J; Molecular Cloning: A Laboratory Manual, 2nd Edn 1989
(18) Spratt, S; J Bacteriol 1981, V146, P1166 HCAPLUS
(19) Taguchi, K; FEMS Microbiol Lett 1999, V176, P183 HCAPLUS
(20) Vlcek, C; Proc Natl Acad Sci USA 1997, V94, P9384 HCAPLUS

L22 ANSWER 25 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 1999:451384 HCAPLUS
DN 131:98488
ED Entered STN: 23 Jul 1999
TI Genetic engineering for the biosynthesis of **medium-chain -length** polyhydroxyalkanoates in plants
IN Poirier, Yves; Mittendorf, Volker
PA Monsanto Company, USA
SO PCT Int. Appl., 128 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM C12N015-82
ICS C12N015-52; C12N015-62; C12N015-81; C12P007-62; A01H005-00; C08G063-06
CC 3-2 (Biochemical Genetics)
Section cross-reference(s): 7, 10, 11, 35

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9935278	A1	19990715	WO 1998-US83	19980105

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

AU 9859071		A1	19990726	AU 1998-59071	19980105
EP 1044278		A1	20001018	EP 1998-902393	19980105
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI					
PRAI WO 1998-US83		A	19980105		

CLASS

PATENT NO.	CLASS	PATENT FAMILY CLASSIFICATION CODES
WO 9935278	ICM	C12N015-82
	ICS	C12N015-52; C12N015-62; C12N015-81; C12P007-62; A01H005-00; C08G063-06

AB Nucleic acids, proteins, and methods for the biosynthesis of polyhydroxyalkanoate polymer materials are disclosed. In a preferred embodiment, expression of a polyhydroxyalkanoate synthase protein with a peroxisome targeting peptide results in the biosynthesis of **medium -chain-length** polyhydroxyalkanoates. Thus, the PHAC1 or PHAC2 polyhydroxyalkanoate synthase subunits from Pseudomonas aeruginosa are fused with peroxisome-targeting regions of isocitrate lyase from Brassica napus for expression in recombinant Arabidopsis thaliana. Localization in the peroxisomes allow for the utilization of intermediates from the lipid β-oxidation pathway. Plants expressing the P. aeruginosa polyhydroxyalkanoate synthase modified for peroxisome targeting produce PHA containing saturated and unsatd. 3-hydroxyalkanoic acids ranging from 6 to 16 carbons. Polyhydroxyalkanoate granules are found within the glyoxysomes or leaf-type peroxisomes of dark- and light-grown plants, resp., as well as in the vacuoles. In an alternative embodiment, exogenous addition of fatty acids to a plant or cell containing a peroxisome targeted polyhydroxyalkanoate synthase protein leads to the biosynthesis of novel polymeric materials. Inclusion of recombinant acyl-**ACP thioesterase**, fatty acyl hydroxylase, yeast multifunctional protein (MFP), and/or hydroxyacyl-CoA epimerase can enhance the production of polyhydroxyalkanoates.

ST polyhydroxyalkanoate biosynthesis plant genetic engineering; Pseudomonas polyhydroxyalkanoate synthase genetic engineering plant; peroxisome targeting polyhydroxyalkanoate synthase plant

IT Enzymes, biological studies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(MFP (multifunctional protein; genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)

IT Cuphea lanceolata
(acyl-**ACP thioesterase** from; genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)

IT Lesquerella
(fatty acyl hydroxylase from; genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)

IT Alfalfa (Medicago sativa)
Arabidopsis thaliana
Banana (Musa)
Barley
Bean (Phaseolus vulgaris)
Cabbage
Canola
Capsicum
Carrot

Castor bean
Celery (Apium graveolens)
Clover (Trifolium)
Coconut (Cocos nucifera)
Corn
Cotton
Cucumber (Cucumis sativus)
DNA sequences
Flaxseed
Genetic engineering
Glyoxysome
Olive
Palm (Arecaceae)
Parsnip
Pea
Peanut (Arachis hypogaea)
Peroxisome
Plant (Embryophyta)
Potato (Solanum tuberosum)
Protein sequences
Radish (Raphanus sativus)
Rape (plant)
Rapeseed
Rice (Oryza sativa)
Soybean (Glycine max)
Spinach (Spinacia oleracea)
Sunflower
Tobacco
Tomato
Watermelon (Citrullus lanatus)
Wheat
(genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)

IT Promoter (genetic element)
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)

IT Aspergillus niger
Fungi
Fusarium
Saccharomyces cerevisiae
Schizosaccharomyces pombe
Streptoverticillium rimofaciens
(host cell; genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)

IT Polyesters, preparation
RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)
(hydroxycarboxylic acid-based; genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)

IT Brassica napus
(isocitrate lyase from; genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)

IT Candida tropicalis
(multifunctional enzyme MFP from; genetic engineering for the biosynthesis of **medium-chain-length**

polyhydroxyalkanoates in plants)

IT Gene, microbial
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(phaC1; genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)

IT Gene, microbial
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(phaC2; genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)

IT Genetic element
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(polyadenylation signal; genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)

IT Pseudomonas
Pseudomonas aeruginosa
(polyhydroxyalkanoate synthase from; genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)

IT Antibiotic resistance
Herbicide resistance
(selectable marker; genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)

IT Organelle
(vacuole; genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)

IT 121684-05-7, Enzyme (Candida tropicalis clone pHDE2 gene HDE precursor reduced) 148768-98-3 148769-00-0 230623-97-9 230623-98-0 230973-69-0 230973-71-4
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(amino acid sequence; genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)

IT 194813-90-6 230289-13-1
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(amino acid sequence; genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)

IT 124-07-2, Octanoic acid, biological studies 141-22-0, Ricinoleic acid 142-62-1, Hexanoic acid, biological studies 593-39-5, Petroselinic acid 638-53-9, Tridecanoic acid 5963-14-4, 8-Methylnonanoic acid 9005-64-5, Tween 20 9005-65-6, Tween 80 9005-67-8, Tween 60
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(fatty acid composition of PHA in response to; genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)

IT 9045-78-7, Isocitrate lyase 134688-88-3, Polyhydroxyalkanoate synthase
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(genetic engineering for the biosynthesis of **medium-**

chain-length polyhydroxyalkanoates in plants)
IT 1883-13-2DP, 3-Hydroxydodecanoic acid, polyhydroxyalkanoate containing
1961-72-4DP, 3-Hydroxytetradecanoic acid, polyhydroxyalkanoate containing
2398-34-7DP, 3-Hydroxyhexadecanoic acid, polyhydroxyalkanoate containing
10191-24-9DP, 3-Hydroxyhexanoic acid, polyhydroxyalkanoate containing
14292-26-3DP, 3-Hydroxydecanoic acid, polyhydroxyalkanoate containing
14292-27-4DP, 3-Hydroxyoctanoic acid, polyhydroxyalkanoate containing
17369-51-6DP, 4-Hydroxydecanoic acid, polyhydroxyalkanoate containing
17587-29-0DP, 3-Hydroxyheptanoic acid, polyhydroxyalkanoate containing
32602-69-0DP, 3-Hydroxytridecanoic acid, polyhydroxyalkanoate containing
40165-87-5DP, 3-Hydroxynonanoic acid, polyhydroxyalkanoate containing
40165-88-6DP, 3-Hydroxyundecanoic acid, polyhydroxyalkanoate containing
62675-78-9DP, polyhydroxyalkanoate containing 86074-08-0DP,
polyhydroxyalkanoate containing 91277-51-9DP, polyhydroxyalkanoate containing
122753-22-4DP, polyhydroxyalkanoate containing 218607-32-0DP,
polyhydroxyalkanoate containing 218607-33-1DP, polyhydroxyalkanoate
containing
218607-35-3DP, polyhydroxyalkanoate containing 218607-36-4DP,
polyhydroxyalkanoate containing 218607-37-5DP, polyhydroxyalkanoate
containing
218607-38-6DP, polyhydroxyalkanoate containing 230949-26-5DP,
polyhydroxyalkanoate containing
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP
(Preparation)
(genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)
IT 9076-73-7, Fatty acyl hydroxylase 68009-83-6, Acyl-**ACP thioesterase** 79986-23-5, 3-Hydroxyacyl-CoA epimerase
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES
(Uses)
(genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)
IT 190894-05-4 230623-51-5 230623-52-6 230623-95-7 230973-64-5
230973-65-6 230973-67-8 230973-68-9 230973-74-7 230973-75-8
230973-76-9
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological
study); USES (Uses)
(nucleotide sequence; genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)
IT 84053-32-7 130488-05-0 162068-02-2 220378-50-7 230289-25-5
230289-37-9 230289-42-6
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES
(Uses)
(peroxisomal targeting peptide; genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)
IT 6379-56-2, Hygromycin 8063-07-8, Kanamycin
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES
(Uses)
(selectable marker; genetic engineering for the biosynthesis of **medium-chain-length** polyhydroxyalkanoates in plants)
RE.CNT 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L22 ANSWER 26 OF 46 WPIDS COPYRIGHT 2004 THE THOMSON CORP on STN
AN 1999-590407 [50] WPIDS
CR 1995-366394 [47]
DNC C1999-172322
TI Modifying the fatty acyl composition of triglycerides in a plant seed.
DC C06 D16
IN DAVIES, H M; HAWKINS, D; LASSNER, M; NELSON, J
PA (CALJ) CALGENE INC
CYC 1
PI US 5968791 A 19991019 (199950)* 71 C12P007-64
ADT US 5968791 A CIP of US 1994-224625 19940406, CIP of US 1994-231196 19940421, CIP of US 1994-254404 19940606, CIP of US 1994-327451 19941021, CIP of WO 1995-US3997 19950331, US 1995-458109 19950601
FDT US 5968791 A CIP of US 5563058
PRAI US 1995-458109 19950601; US 1994-224625 19940406;
US 1994-231196 19940421; US 1994-254404 19940606;
US 1994-327451 19941021; WO 1995-US3997 19950331
IC ICM C12P007-64
ICS C12N009-10
AB US 5968791 A UPAB: 20011211
NOVELTY - Modifying the fatty acyl composition of triglycerides in a plant seed comprises growing a plant that contains a DNA construct for the expression of a foreign plant 1-acylglycerol-3-phosphate acyltransferase protein in its seeds.

DETAILED DESCRIPTION - A method to modify the fatty acyl composition of triglycerides in a plant seed comprises growing a plant to seed that contains a DNA construct for the expression, in the seeds of the plant, of a foreign plant 1-acylglycerol-3-phosphate acyltransferase protein capable of inserting fatty acyl-CoA substrates with a carbon **chain length** of 8-14C or 20C or greater into the sn2 position of a triglyceride.

USE - The method can be used to produce trierucin in the seed oil of a high eruic acid rapeseed plant (claimed). The method is particularly useful for the production of vegetable oils for industrial and food uses. The preferred plants are temperate oilseed crops, in particular rapeseed, sunflower, safflower, cotton, soybean, peanut, coconut and oil palms and corn. It can be used to produce a particular fatty acid in the plant seed

oil and to enhance, control or modify the total fatty acid composition of triglycerides and oils e.g. to enhance the incorporation of laurate into storage oil in rapeseed. The method has applications in genetic engineering to prepare structured plant lipids which contain triacylglycerol molecules with fatty acid groups incorporated into particular positions on the triacylglycerol molecules.
Dwg.0/27
FS CPI
FA AB; DCN
MC CPI: C04-A08C2E; C04-A10G0E; C04-E03E; C04-E08; C04-L04; C10-G02; D05-C; D05-C03D; D05-H12A; D05-H12E; D05-H16B; D05-H17A3

L22 ANSWER 27 OF 46 MEDLINE on STN DUPLICATE 18
AN 2000063345 MEDLINE
DN PubMed ID: 10594123
TI Increased flow of fatty acids toward beta-oxidation in developing seeds of Arabidopsis deficient in diacylglycerol acyltransferase activity or synthesizing **medium-chain-length** fatty acids.
AU Poirier Y; Ventre G; Caldelari D
CS Institut d'Ecologie-Biologie et Physiologie Vegetales, Batiment de Biologie, Universite de Lausanne, CH-1015 Lausanne, Switzerland.. yves.poirier@ie-bpv.unil.ch
SO Plant physiology, (1999 Dec) 121 (4) 1359-66.
Journal code: 0401224. ISSN: 0032-0889.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200002
ED Entered STN: 20000209
Last Updated on STN: 20000209
Entered Medline: 20000203
AB Synthesis of **polyhydroxyalkanoates** (PHAs) from intermediates of fatty acid beta-oxidation was used as a tool to study fatty acid degradation in developing seeds of Arabidopsis. **Transgenic** plants expressing a peroxisomal **PHA** synthase under the control of a napin promoter accumulated **PHA** in developing seeds to a final level of 0. 06 mg g(-1) dry weight. In plants co-expressing a plastidial acyl-acyl carrier protein thioesterase from Cuphea lanceolata and a peroxisomal **PHA** synthase, approximately 18-fold more **PHA** accumulated in developing seeds. The proportion of 3-hydroxydecanoic acid monomer in the **PHA** was strongly increased, indicating a large flow of capric acid toward beta-oxidation. Furthermore, expression of the peroxisomal **PHA** synthase in an Arabidopsis mutant deficient in the enzyme diacylglycerol acyltransferase resulted in a 10-fold increase in **PHA** accumulation in developing seeds. These data indicate that plants can respond to the inadequate incorporation of fatty acids into triacylglycerides by recycling the fatty acids via beta-oxidation and that a considerable flow toward beta-oxidation can occur even in a plant tissue primarily devoted to the accumulation of storage lipids.
CT Check Tags: Support, Non-U.S. Gov't
*Acyltransferases: GE, genetics
*Acyltransferases: ME, metabolism
Arabidopsis: GE, genetics
Arabidopsis: GD, growth & development
*Arabidopsis: PH, physiology
*Fatty Acids, Nonesterified: ME, metabolism

Kinetics
Oxidation-Reduction
Peroxisomes: EN, enzymology
Plants, Genetically Modified: ME, metabolism
Plastids: EN, enzymology
Pseudomonas aeruginosa: EN, enzymology
Pseudomonas aeruginosa: GE, genetics
Seeds: PH, physiology
Thiolester Hydrolases: GE, genetics
*Thiolester Hydrolases: ME, metabolism
CN 0 (Fatty Acids, Nonesterified); EC 2.3. (Acyltransferases); EC 2.3.1.- (poly(3-hydroxyalkanoic acid) synthase); EC 2.3.1.20 (diacylglycerol O-acyltransferase); EC 3.1.2. (Thiolester Hydrolases); EC 3.1.2.14 (oleoyl-(acyl-carrier-protein) hydrolase)

L22 ANSWER 28 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 1999:818100 HCAPLUS
DN 132:149072
ED Entered STN: 29 Dec 1999
TI Testing models of fatty acid transfer and lipid synthesis in spinach leaf using in vivo oxygen-18 labeling
AU Pollard, Mike; Ohlrogge, John
CS Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI, 48824, USA
SO Plant Physiology (1999), 121(4), 1217-1226
CODEN: PLPHAY; ISSN: 0032-0889
PB American Society of Plant Physiologists
DT Journal
LA English
CC 11-2 (Plant Biochemistry)
Section cross-reference(s): 9
AB Oxygen-18 labeling has been applied to the study of plant lipid biosynthesis for the first time. [13C218O2]Acetate was incubated with spinach (Spinacia oleracea) leaves and the 18O content in fatty acid Me esters isolated from different lipid classes measured by gas chromatog.-mass spectrometry. Fatty acids isolated from lipids synthesized within the plastid, such as monogalactosyldiacylglycerol, show an 18O content consistent with the exogenous acetate undergoing a single activation step and with the direct utilization of acyl-acyl carrier protein by the acyl transferases of the chloroplast. In contrast, fatty acids isolated from lipids assembled in the cytosol, such as phosphatidylcholine, show a 50% reduction in the 18O content. This is indicative of export of the fatty acyl groups from the plastid via a free carboxylate anion, and is consistent with the acyl-acyl carrier protein thioesterase:acyl-**CoA synthetase**-mediated export mechanism. If this were not the case and the acyl group was transferred directly from acyl-acyl carrier protein to an acyl acceptor on the cytosolic side, there would be either complete retention of 18O or, less likely, complete loss of 18O, but not a 50% loss of 18O. Thus, existing models for fatty acid transfer from the plastid and for spatially sep. synthesis of "prokaryotic" and "eukaryotic" lipids have both been confirmed.
ST fatty acid transfer lipid formation spinach leaf; oxygen 18 labeling plant lipid formation
IT Proteins, specific or class
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(ACP (acyl-carrier); fatty acid transfer and lipid synthesis in spinach leaf)

IT Cytoplasm
(cytosol; fatty acid transfer and lipid synthesis in spinach leaf)
IT Biological transport
(export; fatty acid transfer and lipid synthesis in spinach leaf)
IT Chloroplast
(fatty acid transfer and lipid synthesis in spinach leaf)
IT Phosphatidylcholines, biological studies
RL: BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
(fatty acid transfer and lipid synthesis in spinach leaf)
IT Isotope effect
(kin isotope effects and discrimination against oxygen-18 in acetic acid metabolism in plant lipid formation)
IT Diglycerides
RL: BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
(monogalactosyl; fatty acid transfer and lipid synthesis in spinach leaf)
IT Metabolism, plant
(oxygen-18 labeling application to study of lipid formation in)
IT Leaf
Spinach (Spinacia oleracea)
(testing models of fatty acid transfer and lipid synthesis in spinach leaf with oxygen-18 labeling)
IT Fatty acids, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PROC (Process)
(testing models of fatty acid transfer and lipid synthesis in spinach leaf with oxygen-18 labeling)
IT Lipids, biological studies
RL: BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
(testing models of fatty acid transfer and lipid synthesis in spinach leaf with oxygen-18 labeling)
IT 9013-18-7, Acyl **CoA synthetase** 68009-83-6, Acyl-**ACP Thioesterase** 77322-37-3, Acyl carrier protein acyltransferase
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(fatty acid transfer and lipid synthesis in spinach leaf)
IT 57-10-3, Hexadecanoic acid, biological studies 60-33-3, Linoleic acid, biological studies 112-80-1, 9-Octadecenoic acid (9Z)-, biological studies 28039-99-8
RL: BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PROC (Process)
(fatty acid transfer and lipid synthesis in spinach leaf)
IT 64-19-7, Acetic acid, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(fatty acid transfer and lipid synthesis in spinach leaf and isotope effect of oxygen-18 in acetic acid metabolism)
IT 257614-64-5P, Acetic-13C2-18O2 acid
RL: BUU (Biological use, unclassified); SPN (Synthetic preparation); BIOL (Biological study); PREP (Preparation); USES (Uses)
(preparation for plant lipid formation study and mass spectrum of)
IT 14797-71-8, Oxygen-18, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU

(Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(testing models of fatty acid transfer and lipid synthesis in spinach leaf with oxygen-18 labeling and kin isotope effect in acetic acid metabolism)

RE.CNT 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD

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(30) Wells, M; Biochim Biophys Acta 1971, V248, P80 HCAPLUS

L22 ANSWER 29 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 19
AN 1999:332383 HCAPLUS
DN 131:141619
ED Entered STN: 31 May 1999
TI A rapid method for detecting bacterial **polyhydroxyalkanoates** in intact cells by Fourier **transform** infrared spectroscopy
AU Hong, K.; Sun, S.; Tian, W.; Chen, G. Q.; Huang, W.
CS Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing, 100084, Peop. Rep. China
SO Applied Microbiology and Biotechnology (1999), 51(4), 523-526
CODEN: AMBIDG; ISSN: 0175-7598
PB Springer-Verlag
DT Journal
LA English
CC 9-5 (Biochemical Methods)
Section cross-reference(s): 10
AB **Polyhydroxyalkanoates** (**PHA**) are synthesized by many bacteria as inclusion bodies, and their biodegradability and structural diversity have been studied with a view to their potential application as biodegradable materials. In this paper, Fourier-**transform** IR spectroscopy (FT-IR) was used to carry out rapid qual. anal. of

PHA in intact bacterial cells. The FT-IR spectra of pure **PHA** containing short-chain-length monomers, such as hydroxybutyrate (HB), **medium-chain-length** hydroxyalkanoate (mclHA) monomers including hydroxyoctanoate (HO) and hydroxydecanoate (HD), or both HB and mclHA monomers, showed their strong characteristic band at 1728 cm-1, 1740 cm-1 or 1732 cm-1 resp. Other accompanying bands near 1280 cm-1 and 1165 cm-1 helped identify the types of **PHA**. The intensity of the methylene band near 2925 cm-1 provided addnl. information for **PHA** characterization. In comparison, bacterial cells accumulating the above **PHA** also showed strong marker bands at 1732 cm-1, 1744 cm-1 or 1739 cm-1, corresponding to intracellular PHB, mclPHA and P(HB + mclHA) resp. The accompanying bands visible in pure **PHA** were also observable in the intact cells. The FT-IR results were further confirmed by gas chromatog. anal.

ST bacteria **polyhydroxyalkanoate** cell; Fourier **transform** IR spectroscopy

IT IR spectroscopy
(Fourier-**transform**; a rapid method for detecting bacterial **polyhydroxyalkanoates** in intact cells by Fourier **transform** IR spectroscopy)

IT Bacteria (Eubacteria)
Gas chromatography
(a rapid method for detecting bacterial **polyhydroxyalkanoates** in intact cells by Fourier **transform** IR spectroscopy)

IT Carboxylic acids, analysis
RL: ANT (Analyte); ANST (Analytical study)
(esters, Polyhydroxy-; a rapid method for detecting bacterial **polyhydroxyalkanoates** in intact cells by Fourier **transform** IR spectroscopy)

IT 1320-61-2, Hydroxybutyrate 85791-94-2 92348-62-4
RL: ANT (Analyte); ANST (Analytical study)
(a rapid method for detecting bacterial **polyhydroxyalkanoates** in intact cells by Fourier **transform** IR spectroscopy)

RE.CNT 17 THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS RECORD

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L22 ANSWER 30 OF 46 MEDLINE on STN DUPLICATE 20
AN 2000037388 MEDLINE
DN PubMed ID: 10570798

TI Establishment of a gene transfer system for Rhodococcus opacus PD630 based on electroporation and its application for recombinant biosynthesis of poly(3-hydroxyalkanoic acids).
AU Kalscheuer R; Arenskotter M; Steinbuchel A
CS Institut fur Mikrobiologie, Westfalische Wilhelms-Universitat Munster, Germany.
SO Applied microbiology and biotechnology, (1999 Oct) 52 (4) 508-15. Journal code: 8406612. ISSN: 0175-7598.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199912
ED Entered STN: 20000113
Last Updated on STN: 20030410
Entered Medline: 19991209
AB A gene transfer system for Rhodococcus opacus PD630 based on electroporation was established and optimized employing the Escherichia coli-Rhodococcus shuttle vectors pNC9501 and pNC9503 as well as the E. coli-Corynebacterium glutamicum shuttle vector pJC1 as suitable cloning vectors for R. opacus PD630, resulting in **transformation** efficiencies up to 1.5 x 10(5) CFUs/microgram plasmid DNA. Applying the optimized electroporation protocol to the pNC9501-derivatives pAK68 and pAK71 harboring the entire PHB synthesis operon from Ralstonia eutropha and the **PHA** synthase gene phaC1 from Pseudomonas aeruginosa, respectively, recombinant **PHA** biosynthesis was established in R. opacus PD630 and the TAG-negative mutant ROM34. Plasmid pAK68 enabled synthesis and accumulation of poly(3HB) in R. opacus PD630 and ROM34 during cultivation under storage conditions from 1% (w/v) gluconate, of poly(3HB-co-3HV) from 0.2% (w/v) propionate and of poly(3HV) from 0.1% (w/v) valerate. Under storage conditions, recombinant strains of PD630 and ROM34 harboring pAK71 were able to synthesize and accumulate **PHA** of the **medium chain length** hydroxyalkanoic acids 3HHx, 3HO, 3HD and 3HDD from 0.1% (w/v) hexadecane or octadecane and a copolyester composed of 3HHp, 3HN and 3HUD from 0.1% (w/v) pentadecane or heptadecane. In the recombinant strains of PD630 and ROM34, the thiostrepton-induced overexpression of a 20 kDa protein was observed with its N-terminus exhibiting a homology of 60% identical amino acids to TipA from Streptomyces lividans.
CT Check Tags: Support, Non-U.S. Gov't
*Acyltransferases: BI, biosynthesis
Acyltransferases: GE, genetics
Amino Acid Sequence
Bacterial Proteins: AN, analysis
Bacterial Proteins: GE, genetics
Bacterial Proteins: ME, metabolism
Chromatography, Gas
Culture Media: CH, chemistry
Electrophoresis, Polyacrylamide Gel
*Electroporation: MT, methods
*Gene Transfer Techniques
Genes, Bacterial
*Genetic Vectors: AD, administration & dosage
Molecular Sequence Data
Mutation
Pseudomonas aeruginosa: EN, enzymology
Pseudomonas aeruginosa: GE, genetics
Recombinant Proteins: BI, biosynthesis
*Rhodococcus: GE, genetics

Rhodococcus: ME, metabolism
Thiostrepton: AD, administration & dosage
*Trans-Activators
Triglycerides: DF, deficiency
RN 1393-48-2 (Thiostrepton)
CN 0 (Bacterial Proteins); 0 (Culture Media); 0 (Genetic Vectors); 0 (Recombinant Proteins); 0 (Trans-Activators); 0 (Triglycerides); 0 (tipA protein, Streptomyces); EC 2.3. (Acyltransferases); EC 2.3.1.- (poly(3-hydroxyalkenoate)polymerase)

L22 ANSWER 31 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 21
AN 1998:745243 HCAPLUS
DN 130:78734
ED Entered STN: 25 Nov 1998
TI Synthesis of **medium-chain-length polyhydroxyalkanoates** in Arabidopsis thaliana using intermediates of peroxisomal fatty acid β-oxidation
AU Mittendorf, Volker; Robertson, Elizabeth J.; Leech, Rachel M.; Kruger, Niels; Steinbuchel, Alexander; Poirier, Yves
CS Institut Biologie Physiologie Vegetales, Batiment Biologie, Universite Lausanne, Lausanne, CH-1015, Switz.
SO Proceedings of the National Academy of Sciences of the United States of America (1998), 95(23), 13397-13402
CODEN: PNASA6; ISSN: 0027-8424
PB National Academy of Sciences
DT Journal
LA English
CC 11-2 (Plant Biochemistry)
AB **Polyhydroxyalkanoate** (**PHA**) is a family of polymers composed primarily of R-3-hydroxyalkanoic acids. These polymers have properties of biodegradable thermoplastics and elastomers. **Medium -chain-length** PHAs (MCL-PHAs) are synthesized in bacteria by using intermediates of the β-oxidation of alkanoic acids. To assess the feasibility of producing MCL-PHAs in plants, Arabidopsis thaliana was **transformed** with the PhaC1 synthase from Pseudomonas aeruginosa modified for peroxisome targeting by addition of the carboxyl 34 amino acids from the Brassica napus isocitrate lyase. Immunocytochem. demonstrated that the modified **PHA** synthase was appropriately targeted to leaf-type peroxisomes in light-grown plants and glyoxysomes in dark-grown plants. Plants expressing the **PHA** synthase accumulated electron-lucent inclusions in the glyoxysomes and leaf-type peroxisomes, as well as in the vacuole. These inclusions were similar to bacterial **PHA** inclusions. Anal. of plant exts. by GC and mass spectrometry demonstrated the presence of MCL-**PHA** in **transgenic** plants to approx. 4 mg per g of dry weight The plant **PHA** contained saturated and unsatd. 3-hydroxyalkanoic acids ranging from 6 to 16 carbons with 41% of the monomers being 3-hydroxyoctanoic acid and 3-hydroxyoctenoic acid. These results indicate that the β-oxidation of plant fatty acids can generate a broad range of R-3-hydroxyacyl-CoA intermediates that can be used to synthesize MCL-PHAs.
ST **polyhydroxyalkanoate** formation **transgenic** Arabidopsis peroxisome beta oxidn
IT Glyoxysome
Peroxisome
Transformation, genetic
(**medium-chain-length polyhydroxyalkanoates** formation in Arabidopsis thaliana using intermediates of peroxisomal fatty acid β-oxidation)
IT Gene, microbial

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(phaC1; **medium-chain-length polyhydroxyalkanoates** formation in Arabidopsis thaliana using intermediates of peroxisomal fatty acid β-oxidation)

IT Arabidopsis thaliana
(**transgenic; medium-chain-length polyhydroxyalkanoates** formation in Arabidopsis thaliana using intermediates of peroxisomal fatty acid β-oxidation)

IT Oxidation
(β-; **medium-chain-length polyhydroxyalkanoates** formation in Arabidopsis thaliana using intermediates of peroxisomal fatty acid β-oxidation)

IT 2398-34-7, 3-Hydroxyhexadecanoic acid 10191-24-9, 3-Hydroxyhexanoic acid 14292-26-3, 3-Hydroxydecanoic acid 14292-27-4, 3-Hydroxyoctanoic acid 32602-69-0, 3-Hydroxytridecanoic acid 40165-87-5, 3-Hydroxynonanoic acid 40165-88-6, 3-Hydroxyundecanoic acid 86074-08-0 122753-22-4 218607-32-0 218607-33-1 218607-35-3 218607-36-4 218607-37-5 218607-38-6 218607-39-7
RL: BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
(**medium-chain-length polyhydroxyalkanoates** formation in Arabidopsis thaliana using intermediates of peroxisomal fatty acid β-oxidation)

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L22 ANSWER 32 OF 46 MEDLINE on STN DUPLICATE 22
AN 1998345982 MEDLINE
DN PubMed ID: 9681004
TI A Cuphea beta-ketoacyl-ACP synthase shifts the synthesis of fatty acids towards shorter chains in Arabidopsis seeds expressing Cuphea FatB thioesterases.
AU Leonard J M; Knapp S J; Slabaugh M B
CS Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331, USA.
SO Plant journal : for cell and molecular biology, (1998 Mar) 13 (5) 621-8. Journal code: 9207397. ISSN: 0960-7412.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199808
ED Entered STN: 19980828
Last Updated on STN: 19980828
Entered Medline: 19980817
AB Acyl-acyl carrier protein (**ACP**) **thioesterases** with specificities on **medium chain** substrates (C8-C14) are requisite enzymes in plants that produce 8:0, 10:0, 12:0 and 14:0 seed oils, but they may not be the sole enzymatic determinants of **chain length**. The contribution to **chain length** regulation of a beta-ketoacyl-ACP synthase, Cw KAS A1, derived from Cuphea wrightii, a species that accumulates 30% 10:0 and 54% 12:0 in seed oils, was investigated. Expression of Cw KAS A1 in Arabidopsis seeds reduced 16:0 from 8.2 to 6.2 mol%, suggesting a KAS II-type activity. In the presence of the KAS I inhibitor cerulenin, however, transgenic seed extracts extended 6:0- and 8:0-ACP at a rate four- to fivefold greater than extracts from untransformed plants, whereas no difference was observed in extension of 14:0- and 16:0-ACP. The effect of KAS A1 on seed oils was tested by combining it with the C. wrightii **medium chain**-specific thioesterases, Cw FatB1 and Cw FatB2, in crosses of transformed plants. Fatty acid synthesis thesis shifted towards shorter chains in progeny expressing both classes of enzymes. KasA1/FatB1 homozygotes produced threefold more 12:0 than the FatB1 parent while 14:0 and 16:0 were reduced by one-third and one-half, respectively. F2 progeny expressing KasA1 and FatB2 produced twofold more 10:0 and 1.4-fold more 12:0 than the FatB2 parent, and the double-transgenic progeny produced one-quarter less 14:0 and one-half less 16:0 than the FatB2 parent. It is hypothesized that the shift towards production of shorter chains resulted from increased pools of **medium chain** acyl-ACP resulting from KAS A1 activity. The combined activities of KAS A1 and FatB thioesterases appear to determine the C. wrightii phenotype.
CT Check Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, Non-P.H.S.
3-Oxoacyl-(Acyl-Carrier-Protein) Synthase: GE, genetics
*3-Oxoacyl-(Acyl-Carrier-Protein) Synthase: ME, metabolism
Arabidopsis: EN, enzymology
Arabidopsis: GE, genetics
*Arabidopsis: ME, metabolism
*Fatty Acids: BI, biosynthesis
Fatty Acids: CH, chemistry
Gene Expression
Genes, Plant
Isoenzymes: GE, genetics

*Isoenzymes: ME, metabolism
Plants, Genetically Modified
Seeds: EN, enzymology
Substrate Specificity
Thiolester Hydrolases: GE, genetics
*Thiolester Hydrolases: ME, metabolism
CN 0 (Fatty Acids); 0 (Isoenzymes); EC 2.3.1.- (beta-ketoacyl-acyl carrier protein synthase I); EC 2.3.1.41 (3-Oxoacyl-(Acyl-Carrier-Protein) Synthase); EC 3.1.2. (Thiolester Hydrolases); EC 3.1.2.- (FATB1 protein, plant)

L22 ANSWER 33 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 1999:39631 HCAPLUS
DN 130:222145
ED Entered STN: 20 Jan 1999
TI **Transgenic** plants for the production of **polyhydroxyalkanoates**, a family of biodegradable thermoplastics and elastomers
AU Poirier, Yves; Nawrath, Christiane
CS Institut de Biologie et Physiologie Vegetales, Batiment de Biologie, Universite de Lausanne, Lausanne, CH-1015, Switz.
SO Transgenic Plant Research (1998), 201-218. Editor(s): Lindsey, Keith. Publisher: Harwood, Amsterdam, Neth.
CODEN: 67FAAE
DT Conference; General Review
LA English
CC 16-0 (Fermentation and Bioindustrial Chemistry)
Section cross-reference(s): 3, 11
AB A review with 82 refs. **Polyhydroxyalkanoates** (PHAs) are a family of polyesters produced in bacteria as a carbon and energy reserve. Depending on their chemical structure, PHAs can have properties ranging from stiff and brittle plastics to elastomers and rubbers. These polymers are completely biodegradable in the environment, being metabolized by microorganisms to carbon dioxide and water. There is a growing interest in using PHAs to replace synthetic plastics in consumer products. Although bacterial fermentation has been used to produce PHAs on a small industrial scale, costs associated with fermentation are too high, making the biol. polymer too expensive in comparison to synthetic plastics. In view of producing PHAs on a large scale and at low cost, the possibility of producing these polymers in plants was explored. The poly-hydroxybutyrate (PHB) biosynthetic pathway has been created in the plant Arabidopsis thaliana using genes from the bacterium Alcaligenes eutrophus. Expression of the PHB pathway in the cytoplasm of plant cells led to a low level of PHB accumulation and was deleterious to plant growth. In contrast, expression of the PHB pathway in the plastid led to PHB accumulation of up to 14% of leaf dry weight, with no significant effects on plant growth. Synthesis of PHB in Arabidopsis opened the way for production of PHAs in crop plants. The range of PHAs synthesized in plants has recently been expanded to include small-**chain-length** and **medium-chain-length PHA** copolymers. Further progress on the production of PHAs in crops, representing a variety of plastic properties, will require synergism between knowledge of the enzymes and genes involved in **PHA** synthesis in bacteria, and engineering of plant metabolic pathways.
ST review polyester manuf **transgenosis** plant
IT Plant (Embryophyta)
Transformation, genetic
(**transgenic** plants for production of

polyhydroxyalkanoates)
IT Polyesters, preparation
RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(**transgenic** plants for production of **polyhydroxyalkanoates**)
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L22 ANSWER 34 OF 46 MEDLINE on STN DUPLICATE 23
AN 97336322 MEDLINE
DN PubMed ID: 9193098
TI Broad-range and binary-range acyl-acyl-carrier protein thioesterases suggest an alternative mechanism for **medium-chain** production in seeds.
AU Voelker T A; Jones A; Cranmer A M; Davies H.M; Knutzon D S

CS Calgene, Inc., Davis, California 95616, USA.. tvoelker@ccmail.calgene.com
SO Plant physiology, (1997 Jun) 114 (2) 669-77.
Journal code: 0401224. ISSN: 0032-0889.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-U65642; GENBANK-U65643; GENBANK-U65644
EM 199707
ED Entered STN: 19970812
Last Updated on STN: 19990129
Entered Medline: 19970728
AB In the current model of **medium-chain** (C8-14) fatty acid biosynthesis in seeds, specialized FatB acyl-acyl-carrier-protein (**ACP**) **thioesterases** are responsible for the production of medium chains. We have isolated and characterized FatB cDNAs from the maturing seeds of elm (Ulmus americana) and nutmeg (Myristica fragrans), which accumulate predominantly caprate (10:0)- and myristate (14:0)-containing oils, respectively. In neither species were we able to find cDNAs encoding enzymes specialized for these **chain lengths**. Nutmeg FatB hydrolyses C14-18 substrates in vitro and expression in Brassica napus seeds leads to an oil enriched in C14-18 saturates. Elm FatB1 displays a binary specificity: one activity is centered on 10:0-ACP, and a second is centered on palmitate (16:0)-ACP. After expression in B. napus seeds the oil is enriched in C10-18 saturates, predominantly 16:0, 14:0, and 10:0. The composition of free fatty acids produced by elm FatB1 in Escherichia coli shifts from C14-16 to mostly C8-10 by increasing the rate of chain termination by this enzyme. These results suggest the existence of an alternative mechanism used in the evolution of **medium-chain** production, a model of which is presented.
CT Check Tags: Comparative Study
Amino Acid Sequence
DNA, Complementary: GE, genetics
Escherichia coli: GE, genetics
*Fatty Acids: BI, biosynthesis
Gene Library
Genetic Engineering
Molecular Sequence Data
Plant Proteins: GE, genetics
*Plant Proteins: ME, metabolism
Recombinant Proteins: ME, metabolism
*Seeds: EN, enzymology
Sequence Analysis, DNA
Sequence Homology, Amino Acid
Species Specificity
Spices
Substrate Specificity
Thiolester Hydrolases: GE, genetics
*Thiolester Hydrolases: ME, metabolism
Trees: EN, enzymology
Trees: GE, genetics
CN 0 (DNA, Complementary); 0 (Fatty Acids); 0 (Plant Proteins); 0 (Recombinant Proteins); EC 3.1.2. (Thiolester Hydrolases); EC 3.1.2.- (FATB1 protein, plant); EC 3.1.2.14 (oleoyl-(acyl-carrier-protein) hydrolase)

L22 ANSWER 35 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 24
AN 1997:513862 HCAPLUS

DN 127:231003
ED Entered STN: 13 Aug 1997
TI Cuphea wrightii thioesterases have unexpected broad specificities on saturated fatty acids
AU Leonard, Jeffrey M.; Slabaugh, Mary B.; Knapp, Steven J.
CS Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR, 97331, USA
SO Plant Molecular Biology (1997), 34(4), 669-679
CODEN: PMBIDB; ISSN: 0167-4412
PB Kluwer
DT Journal
LA English
CC 7-2 (Enzymes)
Section cross-reference(s): 3, 11
AB Cuphea wrightii A. Gray is an herbaceous annual that accumulates 30% caprate (10:0) and 54% laurate (12:0) in seed storage lipids. We investigated the role of acyl-acyl carrier protein (**ACP**) **thioesterases** (TE) in acyl **chain-length** regulation in C. wrightii. Two embryo-derived cDNAs, encoding the TEs Cw FatB1 and Cw FatB2, were isolated. Both proteins were detected in developing embryos and mature seeds but not in other tissues, suggesting involvement in seed oil synthesis. Although expected to be 10:0/12:0-ACP-specific, these genes produced a broad range of fatty acids (12:0, 14:0, and 16:0) in transgenic Arabidopsis with the greatest accumulation at 14:0. Cw FatB2 transformants also accumulated small amts. of 10:0. Because C. wrightii accumulates only .apprx.5% 14:0 and .apprx.2% 16:0, we tested the possibility that gene dosage effects might significantly alter the overall kinetics of the pathway. Phenotypic comparisons of progeny segregating for the transgenes individually and in a hybrid population demonstrated that increased enzyme pools in vivo had a minor effect on diverting fatty acid production to shorter chains. We propose that Cw FatB1 and Cw FatB2 may be necessary but not sufficient determinants of the C. wrightii phenotype.
ST Cuphea acyl **ACP thioesterase** FatB sequence; mRNA gene FatB thioesterase Cuphea seed; **medium chain** fatty acid thioesterase Cuphea; mol evolution acyl **ACP thioesterase** Cuphea
IT Cuphea wrightii
Embryo, plant
Growth and development, plant
Population genetics
Protein sequences
Seed
Transformation, genetic
cDNA sequences
(Cuphea wrightii thioesterases have unexpected broad specificities on saturated fatty acids)
IT mRNA
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
(Cuphea wrightii thioesterases have unexpected broad specificities on saturated fatty acids)
IT Gene, plant
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
(FatB1; Cuphea wrightii thioesterases have unexpected broad specificities on saturated fatty acids)
IT Gene, plant
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP

(Properties); BIOL (Biological study); OCCU (Occurrence)
(FatB2; Cuphea wrightii thioesterases have unexpected broad specificities on saturated fatty acids)

IT Arabidopsis
(expression in; Cuphea wrightii thioesterases have unexpected broad specificities on saturated fatty acids)

IT Fatty acids, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(**medium-chain**; Cuphea wrightii thioesterases have unexpected broad specificities on saturated fatty acids)

IT Evolution
(mol.; Cuphea wrightii thioesterases have unexpected broad specificities on saturated fatty acids)

IT 68009-83-6, Acyl-**ACP thioesterase**
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(Cuphea wrightii **thioesterases** have unexpected broad specificities on saturated fatty acids)

IT 57-10-3, Hexadecanoic acid, biological studies 57-11-4, Octadecanoic acid, biological studies 143-07-7, Dodecanoic acid, biological studies 334-48-5, Decanoic acid 506-30-9, Eicosanoic acid 544-63-8, Tetradecanoic acid, biological studies 27104-13-8 27213-43-0 28933-89-3 28984-77-2
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(Cuphea wrightii thioesterases have unexpected broad specificities on saturated fatty acids)

IT 195160-48-6 195160-49-7
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(amino acid sequence; Cuphea wrightii thioesterases have unexpected broad specificities on saturated fatty acids)

IT 177013-82-0, GenBank U56103 177013-83-1, GenBank U56104
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
(nucleotide sequence; Cuphea wrightii thioesterases have unexpected broad specificities on saturated fatty acids)

L22 ANSWER 36 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:477880 HCAPLUS
DN 129:94481
ED Entered STN: 03 Aug 1998
TI Review on **polyhydroxyalkanoate** formation in the model plant Arabidopsis thaliana
AU Nawrath, Christiane; Poirier, Yves
CS Institut de Biologie Vegetale, Universite de Fribourg, Fribourg, CH-1700, Switz.
SO International Symposium on Bacterial Polyhydroxyalkanoates, Davos, Switz., Aug. 18-23, 1996 (1997), Meeting Date 1996, 119-126. Editor(s): Eggink, Gerrit. Publisher: National Research Council of Canada, Ottawa, Ont. CODEN: 66KZA5
DT Conference; General Review
LA English
CC 16-0 (Fermentation and Bioindustrial Chemistry)
Section cross-reference(s): 3
AB A review with 28 refs. In order to explore potential alternatives to the

production of **polyhydroxyalkanoates** (PHAs) in bacteria, the enzymes of Alcaligenes eutrophus involved in the synthesis of polyhydroxybutyrate (PHB) have been expressed in the model plant Arabidopsis thaliana. Following the successful production of low amts. of high mol. weight PHB in plants expressing the acetoacetyl-CoA reductase and the PHB synthase in the cytoplasm of Arabidopsis cells, expression of the PHB pathway in the plastids was achieved by modifying the PHB enzymes with plastid targeting signals. This strategy resulted in a significant increase in the formation of PHB in Arabidopsis, with a maximum of 14% of the leaf dry weight The increase in PHB production is most likely due to the higher flux in the plastids of acetyl-CoA, the precursor for PHB synthesis. A detailed study of metabolic fluxes in Arabidopsis plants producing high levels of PHB could help to determine the potential problems and limitations of PHB synthesis in Arabidopsis and could be useful for optimizing strategies for the production of PHB in crop plants. The knowledge on PHB production could also be used for the production of PHAs other than PHB. Apart from PHB, no other PHAs have been produced in an eukaryotic system. Arabidopsis will, therefore, be used as a model system for the production in eukaryotes of more complex PHAs, such as poly(hydroxybutyrate-co-hydroxyvalerate) or **medium -chain-length**-PHAs.

ST review Arabidopsis **transgenosis polyhydroxyalkanoate** manuf

IT Carboxylic acids, preparation
RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(hydroxy, polycarboxylic; **polyhydroxyalkanoate** formation in **transgenic** Arabidopsis thaliana)

IT Arabidopsis thaliana
Transformation, genetic
(**polyhydroxyalkanoate** formation in **transgenic** Arabidopsis thaliana)

RE.CNT 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD

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L22 ANSWER 37 OF 46 WPIDS COPYRIGHT 2004 THE THOMSON CORP on STN
AN 1995-115455 [15] WPIDS
DNN N1995-091081 DNC C1995-052674
TI An acyl-(ACP)-thio esterase DNA of **medium-chain** specificity - isolated from Cuphea lanceolata; for plant transformation to produce C10 0 fatty acids, useful in the prodn of eg cosmetics..
DC C06 D16 P13
IN MARTINI, N; SCHELL, J; TOPFER, R; TOEPFER, R
PA (PLAC) MAX PLANCK GES FOERDERUNG WISSENSCHAFTEN
CYC 20
PI WO 9506740 A2 19950309 (199515)* GE 40 C12N015-82
RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
W: AU CA US
AU 9477398 A 19950322 (199527) C12N015-82
WO 9506740 A3 19950622 (199616) C12N015-82
EP 716708 A1 19960619 (199629) GE C12N015-82
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE
AU 688377 B 19980312 (199822) C12N015-82
US 5910631 A 19990608 (199930) A01H005-00
ADT WO 9506740 A2 WO 1994-EP2935 19940902; AU 9477398 A AU 1994-77398 19940902; WO 9506740 A3 WO 1994-EP2935 19940902; EP 716708 A1 EP 1994-928311 19940902, WO 1994-EP2935 19940902; AU 688377 B AU 1994-77398 19940902; US 5910631 A WO 1994-EP2935 19940902, US 1996-605106 19960923
FDT AU 9477398 A Based on WO 9506740; EP 716708 A1 Based on WO 9506740; AU 688377 B Previous Publ. AU 9477398, Based on WO 9506740; US 5910631 A Based on WO 9506740
PRAI DE 1993-4329828 19930903
REP 8.Jnl.Ref; WO 9116421; WO 9211373; WO 9220236; WO 9410288; WO 9507357
IC ICM A01H005-00; C12N015-82
ICS C12N005-14; C12N015-29; C12N015-52; C12N015-55
AB WO 9506740 A UPAB: 19950425
DNA sequence encoding an acyl-(**ACP**)-**thioesterase** (TE) of a **medium chain length** specificity and derivs thereof are new. ACP-TE pref is isolated from plants, pref from Cuphea lanceolata and is a C10:0-specific enzyme. Also claimed are (i) a genomic clone encoding ACP-Te, (ii) plasmids pNBM99-TEg1 and -TEg16 (DSM8477 and 8478), (iii) a method for the prodn of plants including the above DNA, genomic clones or plasmid, which produce a fatty acid of **medium chain length**, pref capric acid (C10:0) or myristic acid (C14:0), and (iv) plants or their parts produced with (iv).
USE - The DNA may be used to transform plants (rape, oil palms, soya) capable of forming middle chain-specific acyl-[**ACP**]-**thioesterases**. C10:0 fatty acids are the basic materials for eg softeners, pesticides, tensides and cosmetics.
ADVANTAGE - By making available of the relevant enzymes, fatty acids of medium length can be produced in higher yields than using prior art methods of plant breeding or plant extraction
Dwg.8/8
FS CPI GMPI
FA AB; GI; DCN
MC CPI: C04-A0800E; C04-E03E; C04-E08; C10-C04E; D05-H12A; D05-H12E; D05-H16B; D05-H17A3; D08-B; D11-B15

L22 ANSWER 38 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 25

AN 1995:658895 HCAPLUS
DN 123:193545
ED Entered STN: 07 Jul 1995
TI Strategies for the sustainable production of new biodegradable polyesters in plants: a review.
AU van der Leij; Feike R.; Witholt, Bernard
CS Inst. of Biotechnology, ETH-Hoenggerberg, Zurich, 8093, Switz.
SO Canadian Journal of Microbiology (1995), 41(Suppl. 1), 222-38
CODEN: CJMIAZ; ISSN: 0008-4166
PB National Research Council of Canada
DT Journal; General Review
LA English
CC 11-0 (Plant Biochemistry)
AB A review with many refs. on pathways of the production of poly(3-hydroxyalkanoates) (**PHA**) with **medium chain length** monomers in higher plants. On the basis of what is known of the genetics and the biochem. of **PHA** formation in bacteria, and of fatty acid metabolism in various organisms, a number of possibilities for **PHA** production in model plants and in economically important crop plants are listed. Along with the mol. biol. of **PHA** synthesis and fatty acid metabolism, theor. and environmental considerations, metabolic engineering strategies, and plant **transformation** systems, are discussed.
ST review **polyhydroxyalkanoate** prodn plant
IT Plant
(strategies for production of biodegradable polyesters in)
IT Polyesters, biological studies
RL: BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
(hydroxy-containing, strategies for production of biodegradable polyesters in plants)

L22 ANSWER 39 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 1996:699323 HCAPLUS
DN 125:322962
ED Entered STN: 25 Nov 1996
TI Genetic manipulation of plant oil composition
AU Voelker, T. A.
CS Calgene Inc., Davis, CA, USA
SO Induced Mutations and Molecular Techniques for Crop Improvement, Proceedings of an International Symposium on the Use of Induced Mutations and Molecular Techniques for Crop Improvement, Vienna, June 19-23, 1995 (1995), 93-99 Publisher: International Atomic Energy Agency, Vienna, Austria.
CODEN: 63NLAP
DT Conference; General Review
LA English
CC 11-0 (Plant Biochemistry)
Section cross-reference(s): 3
AB A review with 20 refs. To date, many approaches of genetically changing the composition of seed oils have been successful. This demonstrates the plasticity of the biosynthesis machinery for this carbon and energy storage form of plants. In the past, mutagenesis and plant breeding were applied, leading, for example, to the elimination of erucic acid (22:1) from rapeseed oil. Also achieved were changes in the levels of sats. or polyunsaturates in several temperate crops. More recently, directed metabolic engineering of the fatty acid composition, facilitated by the advent

of plant transformation, has become a reality. It is possible to engineer a desired phenotype by suppression of an endogenous gene. For example, the so called antisense suppression of an enzyme acting on the intermediate 18:0-ACP, a desaturase, reduced the fraction of unsatd. fatty acids in canola (94%) down to 60%. Stearate (18:0), normally a minor component of canola oil, became prominent (up to 40%). To produce canola oils with **medium chain** fatty acids (C8-C14, absent from canola oil), genes for **medium chain** specific acyl-**ACP thioesterases** from plants which accumulate such fatty acids were transferred to the rapeseed genome. Seed specific expression of the resp. thioesterases resulted in canola plants with more than 50% **medium** chains of different **chain lengths**. Thioesterase engineering was also used for the production of high palmitate (16:0) canola oil. Thus, the current limited number of examples for metabolic engineering of seed oil composition demonstrates that it might be possible in the future to vary the fatty acid profile of a given oil, as long as the necessary genes are available and the crop plant is amenable to genetic transformation.

ST review plant oil genetic transformation engineering

IT Genetic engineering
Transformation, genetic
(genetic manipulation of plant oil composition)

IT Fats and Glyceridic oils
RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(genetic manipulation of plant oil composition)

L22 ANSWER 40 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 1993:645713 HCAPLUS
DN 119:245713
ED Entered STN: 11 Dec 1993
TI **Medium chain** acyl-ACP hydrolysis activities of developing oilseeds
AU Davies, H. Maelor
CS Calgene Inc., Davis, CA, 95616, USA
SO Phytochemistry (1993), 33(6), 1353-6
CODEN: PYTCAS; ISSN: 0031-9422
DT Journal
LA English
CC 11-2 (Plant Biochemistry)
AB Cell-free prepns. from the seed tissues of three different species that accumulate **medium chain** triglycerides were examined for **medium chain** acyl-ACP hydrolysis. Activities with qual. appropriate **medium chain length** specificities were found, suggesting that a thioesterase has evolved in diverse plant families for the production of C8, C10 and C12 fatty acids. A pathway kinetic argument is presented to explain quant. differences between the thioesterase specificities determined in vitro and the fatty acids accumulated in vivo.

ST acyl **ACP** hydrolysis **thioesterase** oilseed

IT California laurel
Camphor tree
Coconut
(**medium chain** acyl-ACP hydrolysis activity in seeds of)

IT Seed
(**medium chain** acyl-ACP hydrolysis in)

IT Proteins, specific or class

RL: BIOL (Biological study)
(ACP (acyl-carrier protein), **medium chain** acyl-, hydrolysis activities of, in developing oilseeds)
IT Fatty acids, biological studies
RL: FORM (Formation, nonpreparative)
(**medium-chain**, formation of, in developing oilseeds, acyl-ACP hydrolysis activity in relation to)
IT Elm
(U. americana, **medium chain** acyl-ACP hydrolysis activity in seeds of)
IT 58943-36-5, Thioesterase
RL: BIOL (Biological study)
(of developing oilseeds, **medium-chain** fatty acid formation in relation to)

L22 ANSWER 41 OF 46 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
AN 93:723066 SCISEARCH
GA The Genuine Article (R) Number: MJ960
TI **MEDIUM-CHAIN** ACYL-**ACP THIOESTERASE** IS NOT THE EXCLUSIVE ENZYME RESPONSIBLE FOR EARLY **CHAIN-LENGTH** TERMINATION IN **MEDIUM-CHAIN** FATTY-ACID SYNTHESIS
AU SCHUCH R (Reprint); BRUMMEL M; SPENER F
CS UNIV MUNSTER, INST BIOCHEM, WILHELM KLEMM STR 2, D-48149 MUNSTER, GERMANY (Reprint)
CYA GERMANY
SO GRASAS Y ACEITES, (MAR/APR 1993) Vol. 44, No. 2, pp. 126-128. ISSN: 0017-3495.
DT Article; Journal
FS AGRI
LA ENGLISH
REC No References
Keyed
AB With the aim to elucidate the regulating mechanisms involved in the biosynthesis of **medium-chain** fatty acids we investigated the substrate specificity of the beta-ketoacyl-ACP synthases (KAS) in extracts obtained from developing seeds of Cuphea lanceolata, a crop producing up to 90% decanoic acid in seed triacylglycerols. Reactions of beta-ketoacyl-ACP synthases were carried out in absence and presence of cerulenin (100 muM) and started by addition of a primer, either acetyl-CoA or acyl-ACPs of **chain-lengths** varying from C2 to C16. The elongation was monitored by the criterion of incorporation of radioactively labelled malonate from [2-C-14]malonyl-CoA into acyl-ACPs. The reaction products were separated by 2.5 M urea-PAGE, electroblotted onto a PVDF-membrane and visualised by autoradiography. The elongation of each primer was quantitatively evaluated by densitometrically scanning of the autoradiograms. The results show that KAS III of C. lanceolata has a high preference for acetyl-CoA, but can, though in small amounts, catalyse elongation reactions of acyl-ACPs up to C6. Experiments in absence of cerulenin show that in C. lanceolata seed extracts beta-ketoacyl-ACP synthases as a whole hardly elongate C10-ACP, a special feature that can be attributed to a low specificity of KAS I for this substrate.
CC FOOD SCIENCE & TECHNOLOGY; CHEMISTRY, APPLIED
ST Author Keywords: CONDENSING ENZYMES; CUPHEA; CUPHEA-LANCEOLATA; BETA-KETOACYL-ACP SYNTHASES

L22 ANSWER 42 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 1993:599621 HCAPLUS

DN 119:199621
ED Entered STN: 13 Nov 1993
TI Engineering **medium-chain** fatty acid production in oilseeds
AU Davies, H. Maelor; Worrell, Ann C.; Radke, Sharon E.; Hawkins, Deborah J.; DiMento, Johanna; Voelker, Toni A.
CS Calgene Inc., Davis, CA, 95616, USA
SO Seed Oils Future (1992), 155-63. Editor(s): MacKenzie, Samuel L.; Taylor, David C. Publisher: AOCS, Champaign, Ill.
CODEN: 59IFAD
DT Conference
LA English
CC 11-2 (Plant Biochemistry)
Section cross-reference(s): 3
AB There has been much speculation concerning the pathways by which certain species synthesize **medium-chain** fatty acids and partition them into storage, as opposed to structural, lipids. The authors previously presented the first evidence for a mechanism of **medium-chain** production (Pollard, M.R. et al., 1991), namely a **medium-chain** acyl-**ACP thioesterase** which effected premature termination of fatty acid biosynthesis. This novel 12:0-**ACP thioesterase** was discovered in the developing oilseeds of California bay (Umbellularia californica, "bay"), in which 12:0 is the principal fatty acid of the reserve triglycerides. This bay thioesterase was subsequently near-homogeneity (Davies, H.M. et al., 1991) and the corresponding cDNA was cloned. Here, the expression of this cDNA in E. coli and in higher plants is described. The results demonstrate the concept of using a heterologous **medium-chain** acyl-**ACP thioesterase** to modify the **chain length** of fatty acids in an oilseed. In addition to the Arabidopsis phenotypes presented here, preliminary results were obtained, showing the production of similar amts. of 12:0 and 14:0 in Brassica napus engineered with the bay thioesterase. Although the expressed 12:0-**ACP thioesterase** activities were considerably in excess of the native long-chain (18:1-**ACP**) **thioesterase**, and therefore probably in excess of the overall fatty acid biosynthesis activity of the embryos, no more than 30 mol % medium chains (12:0 + 14:0) were obtained. This suggests the potential for more efficient interception of the **medium-chain** acyl-ACPs that would lead to higher 12:0 levels. A more detailed characterization of the Arabidopsis and Brassica oils containing the **medium-chain** fatty acids is also in progress.
ST oilseed **medium chain** fatty acid engineering; genetic engineering oilseed fatty acid
IT Seed
(**medium-chain** fatty acid formation in, of oilseed plants, genetic engineering of)
IT Genetic engineering
(of **medium-chain** fatty acid formation in oilseeds)
IT Fats and Glyceridic oils
RL: BIOL (Biological study)
(of oilseed plants, **medium-chain** fatty acid formation in, genetic engineering of)
IT Fatty acids, biological studies
RL: FORM (Formation, nonpreparative)
(**medium-chain**, formation of, in oilseeds, genetic engineering of)
IT Plant
(oilseed, genetic engineering of **medium-chain** fatty

acid formation in)

L22 ANSWER 43 OF 46 MEDLINE on STN DUPLICATE 26
AN 91112825 MEDLINE
DN PubMed ID: 1989513
TI A specific acyl-**ACP thioesterase** implicated in **medium-chain** fatty acid production in immature cotyledons of Umbellularia californica.
AU Pollard M R; Anderson L; Fan C; Hawkins D J; Davies H M
CS Calgene Inc., Davis, California 95616.
SO Archives of biochemistry and biophysics, (1991 Feb 1) 284 (2) 306-12. Journal code: 0372430. ISSN: 0003-9861.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199102
ED Entered STN: 19910329
Last Updated on STN: 19970203
Entered Medline: 19910228
AB Umbellularia californica (California Bay) seeds accumulate 10:0 and 12:0 as principal reserve fatty acyl groups. An in vitro fatty acid synthesis system from the developing cotyledons produces chiefly 10:0 and 12:0, in approximately the same proportions as the intact tissue. The kinetics of acyl thioester and free fatty acid formation in this system suggest that a **medium-chain** specific acyl-acyl-carrier protein (ACP) hydrolysis mechanism is responsible for the preponderance of **medium-chain** products. A crude extract of the developing cotyledons exhibits hydrolytic activity toward acyl-ACPs, with marked preference for 12:0-ACP and 18:1-ACP in the test series 6:0, 8:0, 10:0, 11:0, 12:0, 14:0, 16:0, and 18:1-ACPs. Partial purification of the 12:0-ACP hydrolytic activity has resulted in its separation from the 18:1-ACP hydrolase(s) and the 12:0-coenzyme A hydrolase(s) that are also present, thereby demonstrating its specificity for the 12-carbon acyl **chain length** and the ACP derivative. During cotyledon development, as the proportion of **medium-chain** to other fatty acyl groups increases, the extractable yield of this activity also increases substantially. Collectively these results suggest a role for this 12-**ACP thioesterase** in **medium-chain** production in vivo.
CT Coenzymes: ME, metabolism
*Fatty Acids: BI, biosynthesis
Hydrolysis
Kinetics
Plants: EN, enzymology
*Plants: ME, metabolism
Substrate Specificity
*Thiolester Hydrolases: ME, metabolism
CN 0 (Coenzymes); 0 (Fatty Acids); EC 3.1.2. (Thiolester Hydrolases); EC 3.1.2.14 (oleoyl-(acyl-carrier-protein) hydrolase)

L22 ANSWER 44 OF 46 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
AN 1990:395033 BIOSIS
DN PREV199039065994; BR39:65994
TI ENZYMOLOGY AND MOLECULAR BIOLOGY OF PLANT LIPID BIOSYNTHESIS.
AU SLABAS A R [Reprint author]
CS UNILEVER, SHARNBROOK
SO Journal of Experimental Botany, (1990) Vol. 41, No. SUPPL, pp. P8-2.

Meeting Info.: 1990 ANNUAL MEETING OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY. J EXP BOT.
CODEN: JEBOA6. ISSN: 0022-0957.
DT Conference; (Meeting)
FS BR
LA ENGLISH
ED Entered STN: 30 Aug 1990
Last Updated on STN: 30 Aug 1990
CC General biology - Symposia, transactions and proceedings 00520
Genetics - Plant 03504
Biochemistry studies - Proteins, peptides and amino acids 10064
Biochemistry studies - Lipids 10066
Enzymes - Physiological studies 10808
Metabolism - Lipids 13006
Plant physiology - Enzymes 51518
Plant physiology - Metabolism 51519
IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics); Genetics; Metabolism
IT Miscellaneous Descriptors
ABSTRACT MAIZE RAPE GENE EXPRESSION ACETYL **COENZYME A** CARBOXYLASE FATTY ACID **SYNTHETASE** BETA KETOREDUCTASE ENOYL REDUCTASE CONDENSING ENZYME I ACYL **ACP THIOESTERASE** DESATURASES
ORGN Classifier
Gramineae 25305
Super Taxa
Monocotyledones; Angiospermae; Spermatophyta; Plantae
Taxa Notes
Angiosperms, Monocots, Plants, Spermatophytes, Vascular Plants
ORGN Classifier
Cruciferae 25880
Super Taxa
Dicotyledones; Angiospermae; Spermatophyta; Plantae
Taxa Notes
Angiosperms, Dicots, Plants, Spermatophytes, Vascular Plants
RN 9023-93-2 (ACETYL COENZYME A CARBOXYLASE)
9045-77-6 (FATTY ACID SYNTHETASE)
9037-80-3 (REDUCTASE)
68009-83-6 (ACYL **ACP THIOESTERASE**)
103843-28-3 (DESATURASES)

L22 ANSWER 45 OF 46 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 1981:136204 HCAPLUS
DN 94:136204
ED Entered STN: 12 May 1984
TI Synthesis of long-chain acyl-CoA in chloroplast envelope membranes
AU Joyard, Jacques; Stumpf, P. K.
CS Dep. Biochem. Biophys., Univ. California, Davis, CA, 95616, USA
SO Developments in Plant Biology (1980), 6(Biog. Funct. Plant Lipids), 73-6
CODEN: DPBID2; ISSN: 0166-2538
DT Journal
LA English
CC 11-2 (Plant Biochemistry)
Section cross-reference(s): 7
AB High activities of acyl **CoA synthetase** (sp. activity 1.5-2.5 μmol/h/mg protein) and acyl CoA thioesterase (0.2-0.4 μmol/h/mg protein) were associated with chloroplast envelope membranes. Acetyl-**CoA synthetase** and acyl **ACP**

thioesterase predominated in the stroma fraction. The role of the enzymes in fatty acid synthesis in chloroplasts is discussed. The envelope acyl-CoA thioesterase had a pH optimum of 9.0 and was inhibited by ATP, CoA and Mg, and by unsat. fatty acids. Acyl **CoA synthetase** had a pH range of 7.0-9,5; max at 8.0. Addition properties of these enzymes are given.

ST acyl CoA synthesis chloroplast

IT Chloroplast
(acyl **CoA synthetase** and thioesterase distribution in)

IT 72-89-9 9013-18-7 37270-64-7 68009-83-6
RL: BIOL (Biological study)
(chloroplasts fraction characterization of)

L22 ANSWER 46 OF 46 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

AN 1979:137740 BIOSIS

DN PREV197967017740; BA67:17740

TI THIO ESTERASE I AND THIO ESTERASE II OF ESCHERICHIA-COLI HYDROLYSIS OF NATIVE ACYLACYL'CARRIER PROTEIN THIO ESTERS.

AU SPENCER A K [Reprint author]; GREENSPAN A D; CRONAN J E JR

CS DEP MOL BIOPHYS BIOCHEM, YALE UNIV SCH MED, NEW HAVEN, CONN 06510, USA

SO Journal of Biological Chemistry, (1978) Vol. 253, No. 17, pp. 5922-5926. CODEN: JBCHA3. ISSN: 0021-9258.

DT Article

FS BA

LA ENGLISH

AB Thioesterases I and II of E. coli hydrolyze the thioester linkages between fatty acid and CoA or acyl carrier protein (ACP). Previous workers had shown that chemically synthesized acyl-ACP substrates were hydrolyzed about 20-fold more slowly than the analogous CoA thioesters, and had suggested that the slower rate might be the result of modifications of the protein moiety which had occurred during the chemical synthesis. A series of acyl-ACP substrates were prepared using acyl-ACP synthetase, a recently discovered enzyme which ligates long chain fatty acids to ACP. These preparations of native acyl-ACP are hydrolyzed much more slowly by both thioesterases than the analogous acyl-CoA or chemically synthesized acyl-ACP thioesters. Km of both **thioesterases** for native palmitoyl-**ACP** are 100-200 μM, a value over 10-fold higher than those reported for palmitoyl-CoA. The Vmax values of both enzymes for native palmitoyl-ACP are much lower than the values measured for palmitoyl-CoA. In the chemical procedure for acylating ACP, the amino groups are acetylated. This modification appears responsible for the differing rates of hydrolysis of native acyl-ACP and the substrates made chemically. Acetylation of native palmitoyl-ACP increases the rate of hydrolysis of this substrate by both thioesterases. This modification of ACP is known to destabilize the tertiary structure of ACP under ionic conditions similar to those utilized in these assays. The greater rate of thioesterase cleavage of the chemically synthesized acyl-ACP over the native acyl-ACP can therefore by attributed to denaturation of the protein moiety of the former thioester.

CC Biochemistry methods - Proteins, peptides and amino acids 10054
Biochemistry studies - General 10060
Biochemistry studies - Proteins, peptides and amino acids 10064
Biochemistry studies - Lipids 10066
Biophysics - General 10502
Biophysics - Molecular properties and macromolecules 10506
Enzymes - General and comparative studies: coenzymes 10802
Enzymes - Methods 10804

Enzymes.- Chemical and physical 10806
Enzymes - Physiological studies 10808
Metabolism - Lipids 13006
Metabolism - Proteins, peptides and amino acids 13012
Physiology and biochemistry of bacteria 31000
Microbiological apparatus, methods and media 32000

IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics); Metabolism; Physiology

IT Miscellaneous Descriptors
FATTY-ACID **COENZYME A** ACYLACYL CARRIER PROTEIN **SYNTHETASE**

ORGN Classifier
Enterobacteriaceae 06702

Super Taxa
Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria; Microorganisms

Taxa Notes
Bacteria, Eubacteria, Microorganisms

RN 9031-56-5 (SYNTHETASE)